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

terry fox cancer research
portfolio 2019

Photo credit: Gail Harvey

general overview

Terry Fox (1958-1981)

Terry Fox has become an inspiration to us all. Terry Fox was 18 when he was diagnosed with cancer and lost one leg to it by amputation. In 1980 he started to run across Canada in his Marathon of Hope to support cancer research. He wrote in his letter seeking support, "I will be ready to achieve

something that for me was once only a distant dream reserved for the world of miracles—to run across Canada to raise money for the fight against cancer. We need your help. The people in cancer clinics all over the world need people who believe in miracles."

He has received many honours and awards and today is recognized by many as Canada's greatest hero. His legacy lives on through The Terry Fox Foundation, the millions of people who participate in Terry Fox Runs around the world, the millions of generous donors worldwide who give to cancer research, and the Terry Fox Research Institute.

The Terry Fox Foundation (TFF) **www.terryfox.org**

The Terry Fox Foundation (TFF) maintains the vision and principles of Terry Fox while raising money for cancer research through the annual Terry Fox Run, National School Run Day and other fundraising initiatives. To date, over $750 million has been raised worldwide for cancer research in Terry Fox's name. The first Terry Fox Run was held in 1981, with The Terry Fox Foundation being created in 1988. Its national headquarters are located in Burnaby, BC and it has offices in 9 provinces.

The Foundation invests in cure-oriented, biomedical research through its flagship program, The Terry Fox New Frontiers Program Project Grants. It also supports capacity-building research through its New Investigator awards. The Foundation research portfolio is managed by The Terry Fox Research Institute and affiliated partners.

The Terry Fox Research Institute launched its Marathon of Hope Cancer Centres network on Friday, April 12, 2019, exactly 39 years after Terry Fox began his Marathon of Hope by dipping his prosthetic leg into the Atlantic Ocean at St. John's harbour. Above, local school children joined researchers, special guests and Terry Foxers in front of the Terry Fox statue in St. John's following the announcement.

The Terry Fox Research Institute (TFRI) www.tfri.ca

The Terry Fox Research Institute (TFRI), established in 2008, invests in world-class, collaborative cancer research teams and partnerships. Together with its research and funding partners, TFRI is working to inspire the transformation of cancer research in this country by bringing together leading cancer research and treatment organizations in Canada and empowering them under the framework of the *Marathon of Hope Cancer Centres Network*. Just as Terry Fox united Canadians with his run and dream to end cancer, the *Marathon of Hope Cancer Centres* will unite our cancer researchers to pursue that same goal with precision medicine.

The Institute disbursed $26.6-million in cancer research programs on behalf of the Terry Fox Foundation in 2018-2019. To allocate its resources, TFRI has international experts evaluate the excellence and the potential for impact of all its research projects. TFRI collaborates with over 90 cancer hospitals and research organizations across Canada. Headquartered in Vancouver, BC, the Institute has six nodes across Canada which interact with regional partners and support the mission and vision of the Institute.

The projects and publications listed in this summary of Terry Fox research represent some of the best cancer research being conducted in Canada. Provided by our project leaders, these scientific summaries describe research funded by the Terry Fox Foundation and our partners. Funding partners are acknowledged for specific projects. We are deeply grateful to the patients who participate in this research, and to our researchers, clinicians, scientists and their staff for their dedication, expertise and commitment to making a difference for all cancer patients.

The Terry Fox Research Institute supports five areas of cancer research:

Terry Fox New Frontiers Program Project Grants These programs support Canadian research teams exploring new frontiers in cancer research through the funding of three or more outstanding, independent research projects around a common theme. An open competition is offered annually through TFRI to select the best program projects for funding.

Terry Fox New Investigator Awards These awards provide a three-year operating grant to independent cancer researchers within the first seven years of their first faculty-level appointment. New Investigators must be sponsored and mentored by an existing Terry Fox or Canadian Institutes of Health Research (CIHR) funded program.

Marathon of Hope Cancer Centres Network / Translational Pan-Canadian Cancer Research* On April 12, 2019, TFRI launched its new initiative, the *Marathon of Hope Cancer Centres Network*, which is aimed at accelerating precision medicine for cancer patients across Canada through the establishment of a network of linked pan-Canadian cancer research centres. In March 2019, the Government of Canada announced in its Budget 2019 support for the network with an investment of $150 million over five years, to be matched by participating foundations and centres. To date three consortia (in BC, Ontario and Quebec) have been established to contribute to the development of the network and two more (representing the Prairies and Atlantic regions) will be added by 2020. The Canada-wide network of designated cancer centres will work together to share knowledge and data and harness technology to advance cancer cures through personalized treatments. The network will unite the top-tier cancer researchers and clinicians throughout Canada. Work is currently ongoing to operationalize the network and additional details will be available in 2020 as plans are finalized. Visit *www.marathonofhopecancercentres.ca*

*Currently TFRI is not accepting new applications for this program.

Terry Fox Cancer Research Training Program TFRI currently supports three research training programs.

Terry Fox International Run Program Grants Globally, The Terry Fox Foundation fundraises through its International Run Program. Funds raised support research projects in countries where the run is held. *www.terryfox.org/InternationalRun*

For more information visit: *www.tfri.ca*

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Terry Fox Research Institute Translational Cancer Research Projects

Natural killer cells: new weapons in the iTNT cache for ovarian cancer immunotherapy?

Terry Fox New Investigator Operating Grant (2019-2022)

Investigator: Jeanette E. Boudreau, Dalhousie University

Mentoring Program: The Terry Fox Translational Research Program: Immunotherapy network (iTNT): targeting ovarian cancer

Scientific Summary: Immunotherapy is revolutionizing cancer treatment, especially among tumours with high mutational burdens and immune cell infiltration. High grade serous ovarian cancer (HGSC) is the most prevalent and fatal gynecologic malignancy, but its high mutational burden has generated optimism that immunotherapy might enhance treatment and extend survival. The immunotherapy network is working toward this goal, testing approaches to invigorate the immune system to combat cancer. My laboratory is studying natural killer (NK) cells as targets for immunotherapy against HGSC. With an innate ability to sense cells with DNA damage or undergoing extensive stress, NK cells are important effectors for preventing cancer development and growth, and known to be important mediators of immunotherapy in other cancers. NK cell function is determined by the balance of incoming activating and inhibitory signals, which are detected through an array of germline-encoded receptors, and calibrated by inter-individual genetic diversity. How NK cells can participate in HGSC control or immunotherapy, and whether they can be used as direct effectors for this purpose is unexplored, and the subject of our investigations.

Overall Goal: The goals of this project are to understand how NK cells interact with HGSC so that NK cell activity against HGSC can be maximized. First, we are developing strategies to measure NK cell infiltration, localization and activity in HGSC using microscopy and flow cytometry. With this data, we plan to determine how NK cell activity can be used to predict the outcomes of HGSC treatment, including treatment with immunotherapy. Since NK cells are major detectors of tumour cells that have lost expression of human leukocyte antigens (HLA), a key escape mechanism from other forms of immunotherapy, a major component of our analysis is investigating variations in genes for HLA processing and presentation, and their impact on NK cell function.

Expected Outcomes: 1) To understand how NK cell infiltration and localization in HGSC is associated with molecular subtypes of the disease, prognosis and treatment efficacy; 2) to understand the characteristics of a successful NK cell response against HGSC including the phenotypes of responding NK cells; and 3) to understand how to equip NK cells for maximal control of HGSC, including HGSC tumours that have lost expression of HLA.

Expected Impact: Despite their known anti-cancer features, NK cells have seldom been the direct targets of immunotherapeutic strategies or considered in their design. Our work is expected to illuminate features of NK cells that may be exploited for successful cancer immunotherapy and identify how NK cells collaborate in cancer control. Understood more fully, NK cells may act as important allies for successful cancer therapies, or be manipulated to be primary mediators for HGSC immunotherapy.

Progress update: Work on this project began in January 2019. We have used publicly available datasets (TCGA) to establish that HGSC, even without treatment, harbours a substantial mutational burden in genes associated with HLA processing and presentation. This implies that HGSC may be targeted by the subset of NK cells that respond to loss of HLA. We are following up on this finding with *in vitro* assays, wherein we can measure the specific activity of NK cell subsets against tumours based on their mutational loads, and recreate the most common mutations to determine their impact on NK cell reactivity.

Work has commenced on our aims to identify NK cell subsets and infiltration into HGSC tumours. Toward a goal of enumerating and characterizing tumour-infiltrating lymphocytes, we are developing and validating antibody staining panels for tumour microarrays and flow cytometry. A trainee from my laboratory, Ms. Sarah Nersesian, has visited the laboratories of two collaborators to gain insight into digital pathology practices and is now mobilizing this knowledge to assess NK cells in HGSC. She has performed her first qualitative analysis of NK cells in tumour sections and is set to commence high-throughput analysis in the coming months.

Toward our goal of identifying the full phenotype of tumour-infiltrating NK cells, we are expanding a flow cytometry panel, from 15 to 29 colours. This is enabled by a new flow cytometer available in the Faculty of Medicine at Dalhousie. This approach will allow for more information to be harvested about NK cells in our primary cancer samples; to this end, we feel that the slight delay will ultimately be justified by the expanded information that we will be able to collect. Once validated, this panel can be used to measure NK cell phenotypes and function in patient samples, with comparisons to molecular analysis and patient outcomes.

List of Key Publications:

1. Nersesian S, Glazebrook H, Toulaney J, Grantham SR, Boudreau JE. Naturally killing the silent killer. *Frontiers in Immunology.* 2019 Aug 9; 10:1782.

Investigating radiation responses of pancreatic tumours, their vasculature and microenvironment using in vivo imaging to identify new treatment strategies

Terry Fox New Investigator Operating Grant (2016-2019)

Investigator: Ralph S. DaCosta, Princess Margaret Cancer Centre, UHN

Mentoring Program: The Terry Fox New Frontiers Program Project Grant in Research Pipeline for Hypoxia-Directed Precision Cancer Medicine

Scientific Summary: Radiotherapy (RT) improves local control, stability of disease progression and pain control in many stages of pancreatic cancer. However, these clinical benefits have been limited by: 1) relatively lengthy therapy, often exceeding 5 to 6 weeks; 2) toxicity due to lack of dose conformality; and 3) the fact that concurrent chemoradiation often precludes full-dose systemic therapy. Recent advances in RT, including image guidance, target delineation (fiducial markers, dose constraints), and motion management (active breathing control, respiratory gating and tracking) enable precise RT, with an accuracy of 1 mm and favourable toxicity rates. Known as stereotactic body radiation therapy (SBRT), this method uses 1-5 hypo-fractioned, high doses (20-60 Gy) and is tolerable, shows excellent promise for local control of locally advanced unresectable PCs, and reduces treatment time and visits. It has been coined the new frontier in pancreatic cancer therapy.

Overall Goal: The project goal is to characterize the spatiotemporal association between single fraction versus hypo-fractioned SBRT and pancreatic tumour (PT) hypoxia orthotopically *in vivo*. Since systemic therapy depends on vascular transport, understanding the effect of SBRT on tumour vasculature and if these changes modify the tumour microenvironment (hypoxia) is important. How SBRT influences tumour cell cycle spatiotemporally will also be investigated. Key technical platforms, animal models, quantitative measures, and collaborations will be established to achieve this goal.

Expected Outcomes: 1) To characterize SBRT-induced PT vascular impairment and its effects on tumour hypoxia; 2) To characterize the effect of SBRT on PT cell hypoxia, study its consequences on metastases and use metformin to maximize its response; 3) To examine the effect of SBRT on tumour cell cycle heterogeneity and dynamics *in vivo* and identify the ideal time for proliferation-dependent systemic therapy.

Impact: The novel combination of small animal micro-irradiation, multimodal microscopy, intravital animal models and multi-reporter PT cell lines represents an opportunity for preclinical study of RT response and hypoxia of orthotopic PTs *in vivo* for next generation SBRT treatments. If successful, this may help define the key mechanisms of SBRT response in locally advanced PT, the determinants of hypoxia and its importance of tumour response, metastases and cell cycle, methods of manipulating the tumour microenvironment using metformin to maximize SBRT response while mitigating metastasis. These findings may guide future combination SBRT + systemic therapies clinical trials to help overcome current cure-limiting features of PT and can also be adapted to other cancers.

List of Key Publications:

Maeda A, Chen Y, Bu J, Mujcic H, Wouters BG, and DaCosta RS. In Vivo Imaging Reveals Significant Tumour Vascular Dysfunction and Increased Tumour Hypoxia-Inducible Factor-1a Expression Induced by High Single-Dose Irradiation in a Pancreatic Tumour Model. *International Journal of Radiation Oncology Biology Physics* 2017, 97(1): 184-94.

Abbasi AZ, Gordijo CR, Amini MA, Maeda A, Rauth AM, DaCosta RS, and Wu XY. Hybrid manganese dioxide nanoparticles potentiate radiation therapy by modulating tumour hypoxia. *Cancer Research 2016*, 76(22): 6643-56.

DaCosta RS, Wilson BC, Veilleux I, Chang JH, Kang J, Yoo Y, and Song TK. System and Method for Multi-Modal in Vivo Imaging. United States patent application US 15/085,380. 2016.

Deciphering metastasis maintenance events in adolescent and young adult sarcomas

Terry Fox New Investigator Operating Grant (2019-2022)

Investigator: Dr. Livia Garzia, Research Institute of the McGill University Health Centre, Faculty of Surgery, Division of Orthopaedic Surgery, McGill University

Mentoring Program: The Terry Fox New Frontiers Program Project Grant "Killing the Hydra: Genetic Dissection of Actionable Targets Required for Maintenance of Metastatic Diseases"

Scientific Summary: Sarcomas affect approximately 200 children, adolescents and young adults in Canada every year. Molecular profiling of different types of sarcomas has revealed the aggressive nature of certain subtypes, characterized by distinctive molecular signatures. CIC-rearranged sarcomas are characterized by an adverse prognosis, due to their high tendency to metastasize and their limited response to neo-adjuvant therapies. Metastases are the major cause of death but very little effort has been put in to profiling metastatic tissue from patients and our knowledge of the mechanisms driving CIC-rearranged sarcoma dissemination is lacking. Young patients' sarcoma cancers are often characterized by a paucity of actionable single nucleotide variant mutations. Instead, they are often driven by highly recurrent genetic events such as gene fusions, accompanied by large scale epigenetic reprogramming which makes it extremely hard to distinguish, based on genomics alone, the drivers of disease progression from passengers.

Overall Goal: To decipher what genes and pathways drive progression of CIC rearranged sarcomas, we will establish an in vivo functional genomics model that leverages a whole genome transposon driven mutagenesis approach. We will transform the cells of origin of CIC-rearranged sarcomas by combining the CIC fusion with a transposon-based somatic mutagenesis system. Together, the oncogenic fusion and somatic mutagenesis will recreate the intra-tumour heterogeneity characteristic of human sarcoma cancers, with more aggressive metastatic clones emerging spontaneously in cells with the right combination of mutations. By incorporating a mouse-adapted chemotherapy protocol to our functional genomics screening, we will mimic sarcoma tumours evolution and gain a unique perspective on the genetic events driving metastasis and relapse.

Expected Outcomes: We will identify candidate driver genes or metastasis and therapy resistance that will be further pursued as potential targets to prevent or treat metastasis. Providing candidates to be explored as targets for precision therapy approaches to prevent or cure metastatis of CIC rearranged sarcomas.

Impact: Most young patients that die of sarcoma succumb to metastatic disease while the primary tumours in the bone or soft tissues are cured. Our project will shed light on the mechanism of metastatic dissemination of sarcomas by creating genetically engineered models that change in space and time, just like human cancers. This project has the potential to change the way metastatic disease is treated, emphasizing prevention and targeted strategies.

Understanding the function of circular RNA in tumour hypoxia

Terry Fox New Investigator Operating Grant (2017-2019)

Investigator: Housheng Hansen He, Princess Margaret Cancer Centre

Mentoring Program: A Research Pipeline for Hypoxia-directed Precision Cancer Medicine

Scientific Summary: The presence of intratumoral hypoxia is strongly associated with poor clinical outcome through its ability to promote conventional therapy resistance and aggressive disease. Development of hypoxia-directed therapy relies on improved understanding of hypoxia signaling and metastasis in cancer. The recent identification of large amounts of cancer-associated noncoding RNAs has made them the new frontier in cancer research. However, their role in tumour hypoxia remains largely unexplored.

Aims: The overall objective of this proposal is to investigate the function of circular RNAs in tumour hypoxia for the purpose of identifying novel biomarkers and therapeutic targets for hypoxia-directed therapy. The central **hypothesis** is that these deregulated circular RNAs in hypoxic tumours regulate hypoxia signaling and create specific vulnerabilities in hypoxic tumour cells. In Aim 1, we will identify hypoxia related circular RNAs in prostate tumours from CPC-GENE and the sponsoring TFRI program. These circular RNAs will be further validated in cell line models under normoxic and hypoxic conditions. In Aim 2, we will perform shRNA lentiviral based functional genomic screens to evaluate the importance of these circular RNAs on cell survival and proliferation under normoxic and hypoxic conditions. The targets will be subsequently validated in Aim 3 through a series of in vitro and in vivo approaches.

Updates: We have completed circular RNA analysis in the CPC-GENE cohort, and have identified more than 25,000 high confident circular RNAs. Amongst them, ~200 are associated with hypoxic tumours. One of the top candidates, circular SChLAP1, regulates hypoxia response through glycolysis and mTOR pathways. Circular SChLAP1 can be detected in high-risk prostate cancer patient blood samples, highlighting its potential to serve as a biomarker for hypoxia in liquid biopsy. We also completed shRNA screens of ~1,500 highly expressed circular RNAs and identified 171 as essential to prostate cancer cell growth in four prostate cancer cell lines.

List of Key Publications:

1) Han D*, Chen S*, Han W, Gao S, Owiredu J, Patalano S, Macoska J, Balk S, He H#, Cai C#. ZBTB7A mediates the transcriptional repression activity of androgen receptor in prostate cancer. Cancer Research. 2019. PMID: 31444154. *IF=9.13*.
2) Chen S*, Huang V*, Xu Xin*, Julie L*, Soares F*, Jeon J, Zeng Y, Hua J, Petricca J, Guo H, Wang M, Yousif F, Zhang Y, Donmez N, Ahmed M, Volik S, Lapuk A, Chua M, Heisler L, Foucal A, Fox N, Fraser M, Bhandari V, Shiah Y, Guan J, Orian M, Picar V, Hovington H, Bergeron A, Lacombe L, Fradet Y, Tetu B, Liu S, Feng F, Wu X, Y Shao, Komor M, Sahinalp C, Collins C, Hoogstrate Y, Jong M, Fijneman R, Fei T, Jenster G, van der Kwat T, Bristow RG, Boutros P#, He H#. Widespread and functional RNA circularization in localized prostate cancer. *Cell*. 2019. PMID: 30735634. IF=30.41.
3) Guo H*, Ci X*, Ahmed M, Hua J, Soares F, Lin D, Puca L, Vosoughi A, Xue H, Li E, Su P, Chen S, Tran N, Liang Y, Zhang Y, Xu X, Xu J, Sheahan A, Ba-Alawi W, Zhang S, Mahamud O, Vellanki RN, Gleave M, Bristow RG, Haibe-Kains B, Poirier J, Rudin C, Tsao MS, Wouters BG, Fazli L, Feng F, Ellis L, Kwast T, Berlin A, Koritzinsky M, Boutros PC, Zoubeidi A, Beltran H, Wang YZ#, He H#. ONECUT2 is a driver of neuroendocrine prostate cancer. *Nature Communications*. 2019. PMID: 30655535. IF=12.35.

Targeting resistant glioblastoma multiforme (rGBM) through suppression of overactive DNA repair activity

Terry Fox New Investigator Operating Grant (2017-2020)

Investigator: Sachin Katyal, University of Manitoba and CancerCare Manitoba

Mentoring Program: The Terry Fox New Frontiers Program Project Grant: Targeting clonal heterogeneity in treatment-refractory glioblastoma with novel and empiric immunotherapies

Scientific Summary: Glioblastoma multiforme (GBM) is a highly aggressive brain cancer that afflicts individuals of all ages. While rare, these tumours are responsible for a significant amount of malignancy-related morbidity and mortality. Treatment of primary GBM (pGBM) usually involves surgical resection of the tumour followed by radiation and chemotherapy (typically temozolomide(TMZ)); however, GBM prognoses are generally poor and rarely curable due to drug resistance (rGBM) and tumour recurrence. As such, there is a critical requirement to improve upon current anti-GBM therapeutic strategies, particularly in resolving rGBM.

We are targeting cellular DNA damage repair pathways to enable existing anti-cancer therapeutic strategies to more specifically kill cancer cells by lowering the radio- and chemotherapeutic threshold of tumour cell genotoxicity. By expanding our current understanding of the tumour's DNA damage repair response, our research findings will enable us to pursue new brain tumour treatment strategies that enhance chemotherapeutic efficacy, patient survival and quality of life. We use innovative approaches to understand tumour DNA repair biology in order to develop refined, targeted and more effective personalized approaches to treat patients stricken with brain tumours. This dynamic, original and iterative research program will elucidate the complex molecular basis by which cancer chemotherapeutics fail to treat recurring secondary tumours thus leading to better strategies to treat these "life-ending" terminal cancer subtypes.

Overall Goal: The purpose of this research study is to identify key DNA repair enzymes that mediate accelerated and efficient repair activity in rGBM. We will then modulate/inhibit these new targets in patient-derived pGBM and rGBM cells to (re) sensitize cells to DNA damaging chemotherapeutics. Furthermore, as each individual GBM patient's tumour is unique and has inherent heterogeneity, we have developed unique one-of-its kind tools that allow us to perform large scale DNA repair analyses and interrogate the entire contingent of FDA-approved drugs to identify personalized treatment combinations to counteract each individual's recurring tumour. These initiatives are highly innovative and novel; bringing forth unique approaches to GBM research and personalized management of brain tumour treatments.

Expected Outcomes: 1. Transcriptomic analysis of DNA repair enzyme expression during pGBM-to-rGBM progression to identify novel targets. 2. Modulation of DNA damage repair targets in rGBM to ameliorate chemoradiotherapy. 3. "Quick-to-clinic" personalized-medicine approach for treating rGBM.

Updates: In conjunction with Dr. Singh and her lab, we have identified four actionable DNA repair targets. Our lab has recently generated a novel, fluorescent-based tractable cell line model of TMZ resistance and has accumulated a number of patient-derived brain tumour-initiating cells (BTICs) with which to test these targets. One target has been validated via qPCR and Western analysis and we are presently pursuing functional analysis in the context of pGBM vs rGBM biology. Another target has illuminated a particular DNA repair pathway's unique role in mediating rGBM resistance to TMZ, accelerated cell growth and rGBM cell motility/invasiveness. We continue to expand our dearth of patient-derived BTICs to test these targets and we are using genetic techniques and small molecule approaches to further validate our findings.

List of Key Publications:

B. Larson, Sinha, A and Katyal, S. , Automated Imaging and Dual-Mask Analysis of γH2AX Foci to Determine DNA Damage on an Individual Cell Basis. (2016). *Biotek Instruments Application Notes.*
B. Larson, Sinha, A and Katyal, S., Automated Comet Assay Imaging and Dual-Mask Analysis to Determine DNA Damage on an Individual Comet Basis. (2016). *Biotek Instruments Application Notes.*
P. Vora, C. Venugopal, N. McFarlane, S. K. Singh. (2015). Culture and Isolation of Brain Tumor Initiating Cells. *Curr Protoc Stem Cell Biol* 34, 3 3 1-13.
*S. Katyal, Lee, Y., Nitish, K., Downing, S., Zhao, J., Li, Y., Russell, H.R., Petrini, J.H.J, Nitiss, J.L., *McKinnon, P.J. (2014). *Nat. Neurosci.* 17: 813-21. * denotes co-corresponding authorship

Dissecting biological heterogeneity in follicular lymphoma into clinically relevant molecular subtypes

Terry Fox New Investigator Award (2018-2020)

Investigator: Robert Kridel, Princess Margaret Cancer Centre, UHN.

Mentoring Program: The Terry Fox New Frontiers Program Project Grant in Overcoming Treatment Failure in Lymphoid Cancers

Scientific Summary: Follicular lymphoma (FL) is the most common indolent lymphoma, with an estimated 2,500 new diagnoses per year in Canada. FL remains largely incurable, despite recent therapeutic advances. Currently, all symptomatic patients are treated with a combination of rituximab and chemotherapy ("immunochemotherapy"). However, outcomes following such treatment are extremely variable. The 20% of patients who experience early progression (< 2 years) after starting immunochemotherapy have poor outcomes, with 50% of such patients dying from lymphoma within five years. On the other hand, patients without early progression have no added mortality compared to the general population. It is currently infeasible to predict poor outcome with sufficient accuracy to change clinical management, which represents a major impediment for clinical research and for improving patient outcomes. The overarching objective of this proposal is to rigorously define and validate molecular subtypes by unravelling inter- and intra-patient heterogeneity. We propose to elucidate the intricate relationships between lymphoma biology and response to treatment, at once providing predictive biomarkers to guide patient management, and highlighting discrete biological contrasts within the heterogeneous molecular landscape that is inherent to FL.

Aims: In Aim 1, we will build on our previous research that identified high expression of the FOXP1 transcription factor as a key determinant of poor outcome following immunochemotherapy. We will identify FOXP1 mRNA isoforms from RNAseq data and define transcriptional networks that are regulated by such isoforms. In Aim 2, we will apply unbiased machine learning to discover novel FL subtypes by joint clustering of RNAseq, DNA sequencing and methylation data in a large cohort of primary patient samples. We will perform pathway analysis in these novel subtypes to identify therapeutic vulnerabilities, and correlate novel subtypes with FOXP1 mRNA expression patterns identified in Aim 1. In Aim 3, we will leverage recent technological advances to perform single cell RNAseq and determine whether the expression profiles seen in novel subtypes identified in Aim 2 are related to tumour, as opposed to normal microenvironment cells.

List of Key Publications:

1. Silva A, Bassim S, Sarkozy C, et al. Convergence of risk prediction models in follicular lymphoma. *Haematologica.* 2019;104(6): e252–e255.
2. Mottok A, Jurinovic V, Farinha P, et al. FOXP1 expression is a prognostic biomarker in follicular lymphoma treated with rituximab and chemotherapy. *Blood.* 2018;131(2):226–235.
3. Kridel R, Chan FC, Mottok A, et al. Histological Transformation and Progression in Follicular Lymphoma: A Clonal Evolution Study. *PLoS* Med. 2016;13(12): e1002197.
4. Pastore A, Jurinovic V, Kridel R, et al. Integration of gene mutations in risk prognostication for patients receiving first-line immunochemotherapy for follicular lymphoma: a retrospective analysis of a prospective clinical trial and validation in a population-based registry. *Lancet. Oncol.* 2015;2045(15):1–12.

Deciphering the oncogenic properties of cancer-associated IDH1/2 mutations

Terry Fox New Investigator Operating Grant (2017-2020)

Investigator: Frédérick A. Mallette, Maisonneuve-Rosemont Hospital Research Centre and Université de Montréal

Mentoring Program: The Terry Fox New Frontiers Program Project Grant in Targeting Metabolic Vulnerabilities in Cancer

Scientific Summary: Gliomas and leukemias are devastating tumours that remain highly refractory to treatment, thus highlighting the need for new and improved therapeutic strategies. Mutations in genes encoding enzymes involved in the metabolism of the Krebs cycle, such as the isocitrate dehydrogenases 1 and 2 (IDH1/2), are frequently identified in astrocytomas and secondary glioblastomas, as well as in acute myeloid leukemias. However, the precise molecular mechanism by which these mutations promote tumourigenesis remains to be fully characterized. Gain-of-function mutations in IDH1/2 have been shown to stimulate production of the oncogenic metabolite 2-hydroxyglutarate (2HG). Interestingly, 2HG acts as an inhibitor of -ketoglutarate (KG)-dependent enzymes including the JUMONJI family of lysines demethylases (KDMs).

Project Aims: We recently characterized the unsuspected link between mutated IDH1/2-2HG and stimulation of mTOR activity (Carbonneau et al. *Nature Communications* 2016). Moreover, a siRNA screen covering a panel of KG-dependent enzymes identified the lysine demethylase KDM4A/JMJD2A as a novel regulator of mTOR. Interestingly, KDM4A directly binds to mTORC1/2 protein complexes, suggesting a novel mode of mTOR regulation. Now we propose that 2HG produced by mutated IDH1/2 acts as an oncometabolite by activating oncogenic cellular signaling. The specific aims are: 1) to determine the molecular mechanisms by which inhibition of KDM4A by 2HG modulates mTOR activity; 2) to define novel functions of DEPTOR, and 3) to identify novel oncogenic pathways regulated by mutated IDH1/2. This research proposal covers a large array of methods and approaches involving genetics, genomics, bioinformatics, as well as cytological, histological and biochemical techniques.

Cancer cells exploit the metabolism differently than their normal counterparts. The proposed research project will investigate a previously unsuspected link between metabolic defects and oncogenic activation of mTOR. The identified mechanism of modulation of mTOR by KDM4A might also represent an important event in promoting transformation in brain, but also non-brain cancers. Elucidating the oncogenic activities of IDH1/2 mutations will permit the development of novel targeted and personalized therapies for deadly brain cancers.

List of Key Publications:

1. Gagné L.M.*, Boulay K.*, Topisirovic I., Huot M.-E.#, Mallette F.A.# (2017). # Co-corresponding authors. Oncogenic activities of IDH1/2 mutations: From epigenetics to cellular signaling. *Trends in Cell Biology.*
2. Oubaha M., Miloudi K., Dejda A., Guber V., Mawambo G., Germain M.-A., Bourdel G., Popovic N., Rezende F., Kaufman R.J., Mallette F.A.#, Sapieha P. #. (2016) # Co-corresponding authors. Senescence-associated secretory phenotype contributes to pathological angiogenesis in retinopathy. *Science Translational Medicine*. 8, 362ra144.
3. Carbonneau M., Germain M.-A., Gagné L.M., Lalonde M.-E., Motorina A., Guiot M.-C., Secco B., Vincent E.E., Tumber A., Hulea L., Bergeman J., Oppermann U., Jones R.G., Laplante M., Topisirovic I., Petrecca K., Huot M.-E.#, Mallette F.A.# (2016) # Co-corresponding authors. The oncometabolite 2-hydroxyglutarate activates the mTOR signaling pathway. *Nature Communications*. 7, 12700.
4. Neault M., Mallette F.A. #, and Richard S. # (2016) miR-137 modulates a tumour suppressor network inducing senescence in pancreatic cancer cells. # Co-corresponding authors. *Cell Reports*, 14, 1966-1978.
5. Jin J., Hu H., Li H.S., Yu J., Xiao Y., Brittain G.C., Zou Q., Cheng X., Mallette F.A., Watowich S.S., Sun S.C. (2014) Noncanonical NF-kB pathway controls the production of type I interferons in antiviral innate immunity. *Immunity* 40, 342-354.
6. Deschênes-Simard X., Gaumont-Leclerc M.-F., Bourdeau V., Lessard F., Moiseeva O., Forest V., Igelmann S., Mallette F.A., Saba-El-Leil M.K., Meloche S., Saad F., Mes-Masson A.-M. and Ferbeyre G. (2013) Tumour suppressor activity of the ERK/MAPK pathway by promoting selective protein degradation. *Genes & Development* 27, 900-915.
7. Mallette F.A., Richard S. (2012) JMJD2A promotes cellular transformation by blocking cellular senescence through transcriptional repression of the tumour suppressor CHD5. *Cell Reports* 2, 1233-1243.
8. Neault M.*, Mallette F.A.*, Vogel G., Michaud-Levesque J., Richard S. (2012) Ablation of PRMT6 reveals a role as a negative transcriptional regulator of the p53 tumour suppressor. *Equal contribution. *Nucleic Acids Research* 40, 9513-21.
9. Mallette F.A., Mattiroli F., Cui G., Young L.C., Hendzel M.J., Mer G., Sixma T.K., Richard S. (2012) RNF8-dependent degradation of KDM4A/JMJD2A triggers 53BP1 recruitment to DNA damage sites. *EMBO Journal* 31, 1865-1878.

Bubble-mediated therapy for metastatic disease in the spinal cord

Terry Fox New Investigator Operating Grant (2019-2021)

Investigator: Meaghan A. O'Reilly, Sunnybrook Research Institute

Mentoring Program: The Terry Fox New Frontiers Program Project Grant in Ultrasound and MRI for Cancer Therapy

Scientific Summary: Leptomeningeal metastases (LM) are a serious complication of cancer that are diagnosed in approximately 5% of patients with solid tumours. Of these, breast cancer accounts for the greatest number of cases. LM occurs when cancer spreads and infiltrates the lining of brain and spinal cord, causing neurological symptoms. There are no effective treatments for LM. Once diagnosed, patient survival is on the order of a few months. These poor outcomes are due largely to the limited ability of drugs to reach these tumours. Ultrasound can enhance drug delivery to tumours by interacting with injectable micro-sized gas spheres (microbubbles) that vibrate in the ultrasound field and (a) stimulate blood vessel walls, and (b) help disperse drugs. These effects can be directed to specific sites without impacting the surrounding tissue. Further, because ultrasound is non-ionizing, this local therapy can be repeated without the concerns for dose limits that exist for radiation therapy. We will develop ultrasound and bubble-mediated therapy for treating LM situated in the spinal cord.

Project Aims: Recently we have shown that the drug-enhancing effects of ultrasound can be achieved in the spinal cord, which could provide a treatment avenue for LM. Building on positive pilot results in HER2+ LM tumours, we will extend our investigations to enhance not only the delivery of drugs from the blood stream to the spinal cord, but also from the cerebrospinal fluid compartment. We will study the impact of ultrasound treatments on LM tumours in the spinal cord of rats, and assess the impact on slowing tumour growth and prolonging survival. In parallel we will develop the clinical-scale approach on an MRI-guided ultrasound phased array appropriate for clinical investigations, and test it in pigs. The specific aims are: 1) To study the efficacy of short-burst ultrasound in enhancing drug delivery to leptomeningeal metastases and study the tumour response to treatment using quantitative MRI; 2) To implement the developed methods on an MRI-guided focused US-phased array and test it in an animal model of clinically relevant scale.

Impact: Currently there are no good treatment options for LM and any treatment is considered palliative. At the end of this grant we expect that our small animal studies will demonstrate improved outcome following ultrasound and bubble-mediated drug delivery. Further, we expect our clinical-scale work to advance this method to readiness for clinical investigations, and we have engaged clinical collaborators with a strong interest in bringing this technology to patients.

List of Key Publications:

1) Fletcher SMP, Ogrodnik N, O'Reilly MA. (2019) Enhanced detection of bubble emissions through the intact spine for monitoring ultrasound-mediated blood-spinal cord barrier opening. doi: 10.1109/TBME.2019.2936972[Ahead of Print]
2) Xu R, O'Reilly MA. (2019) A Spine-Specific Phased Array for Transvertebral Ultrasound Therapy: Design & Simulation. *IEEE Trans Biomed* Eng. doi: 10.1109/TBME.2019.2912146 [Ahead of Print]
3) Fletcher SMP, O'Reilly MA. (2018). Analysis of Multi-Frequency and Phase Keying Strategies for Focusing Ultrasound to the Human Vertebral Canal. *IEEE Trans Ultrason Ferroelectr Freq Control*. 65(12); 2322-2331
4) O'Reilly MA, Chinnery T, Yee ML, Wu SK, Hynynen K, Kerbel RS, Czarnota GJ, Pritchard KI, Sahgal A. (2018). Preliminary Investigation of Focused Ultrasound-Facilitated Drug Delivery for the Treatment of Leptomeningeal Metastases. Scientific reports. 8(1): 9013.

Single cell dissection and non-invasive monitoring of childhood cancer and immune systems during treatment

Terry Fox New Investigator Operating Grant (2017-2020)

Investigator: Trevor Pugh, Princess Margaret Cancer Centre, University Health Network

Mentoring Program: Precision Oncology For Young People (PROFYLE)

Scientific Summary: We seek to understand how the balance of cancer and immune cells in pediatric tumours may dictate the efficacy of a new class of drugs that activate the immune system to combat cancer. We will also assess whether the response of tumour and immune cells to immunotherapy may be tracked using analysis of simple blood draws rather than invasive surgery or tissue biopsy.

Previous Research: Immunotherapies that activate immune cells to attack cancer cells are emerging as a transformational new approach to combat cancer. While 20 to 40% of adult patients have dramatic responses depending on cancer type, there are no predictors of which patients respond and why they eventually become resistant. While initially proven in adult tumours, immunotherapies have also benefitted children with cancer despite significant differences in genetic alterations between pediatric and later-onset cancers. Since tumour masses are a combination of immune, tissue, and cancer cells, we hypothesize that it is the combined interplay of these cells that dictate response to immunotherapy, particularly in childhood cancers with few mutations compared to adults.

Previous Methods: We propose to use cutting-edge, single cell RNA sequencing methods to analyze which genes are expressed by each of thousands of cells from serial collections of tumours from children with cancer. We will also examine the DNA of immune cells that infiltrate tumours to understand how they recognize tumour cells. This will allow us to track the changes in cell type and immune response over time, as well as link specific changes to clinical outcome of each patient. To assess whether these patterns can be monitored using non-invasive blood tests, we will apply a new liquid biopsy sequencing technology to track immune and tumour response in serial blood samples over time.

Impact and Relevance: At the conclusion of the study, we anticipate having a deep understanding of how tumour and non-cancerous cells react to immunotherapy and to nominate additional strategies to help the immune system destroy cancer cells in children. We will also have assessed whether blood testing may be used to track the progress and effect of immunotherapy in lieu of requiring more invasive tumour biopsies, tissue resections, or surgery.

List of Key Publications:

1. CapTCRSeq: A DNA hybrid capture method for assessment of T-cell receptor clonality. Mulder DT, Mahé ER, Dowar M, Hanna Y, Delabie J, Nguyen L, Ohashi PS, Kamel-Reid S, Pugh TJ. In submission.
2. Bamgineer: Introduction of simulated, haplotype-phased and allele-specific copy number variants into exome sequence data sets. Samadian S, Bruce JP, Pugh TJ. In submission. http://biorxiv.org/content/early/2017/03/22/119636
3. AACR Project GENIE: Powering Precision Medicine through an International Consortium. AACR Project GENIE Consortium. Cancer Discovery. 2017 Aug;7(8):818-831. PMID 28572459
4. Therapeutic radiation for childhood cancer drives structural aberrations of NF2 in meningiomas. Agnihotri S*, Suppiah S*, Tonge PD*, Jalali S*, Danesh A, Bruce JP, Mamatjan Y, Klironomos G, Gonen L, Au K, Mansouri S, Karimi S, Sahm F, von Deimling A, Taylor MD, Laperriere NJ, Pugh TJ, Aldape KD+, Zadeh G+ Nature Communications. 2017 Aug 4;8(1):186. PMID: 28775249. (* co-first authors, + co-senior authors)
5. Circulating tumour DNA sequence analysis as an alternative to bone marrow aspirates for clinical management of multiple myeloma Kis O, Kaedbey R, Chow S, Danesh A, Dowar M, Li T, Li ZH, Liu J, Mansour M, Sukhai M, Zhang T, Kamel-Reid S, Trudel S+, Pugh TJ+. Nature Communications. 2017 May 11;8:15086. PMID: 28492226 (+ co-senior authors)
6. Genomic profiling of 1239 diverse pediatric cancers identifies novel discoveries across tumours. Chmielecki J, Bailey M, He J, Elvin J, Vergillio J, Ramkissoon S, Suh J, Frampton GM, Morley S, Spritz D, Ali S, Gay L, Erbach RL, Ross JS, Buxhaku J, Davies H, Faso V, Germain A, Glanville B, Miller VA, Stephens PJ, Janeway K, Maris J, Meshinchi S, Pugh TJ, Shern JF, Lipson D. Cancer Research. 2017 Jan 15;77(2):509-519.
7. A Novel Innate Lymphoid Cell Population Regulates Tumour-Associated T cells. Crome SQ, …, Pugh TJ, Lanier LL, Ohashi PS. Nature Medicine. 2017 Mar;23(3):368-375. PMID 28165478.
8. Exome sequencing of pleuropulmonary blastoma reveals frequent biallelic loss of TP53 and two-hits in DICER1 resulting in retention of 5p-derived miRNA hairpin loop sequences. Pugh TJ, …, Getz G, Hoffher S, Messinger YH, Dehner LP, Roberts CMW, Rodriguez-Galindo C, Williams GW, Rossi CT, Meyerson M, Hill DA. Oncogene. 2014 Nov 6;33(45):5295-302. PMID 24909177.
9. The genetic landscape of high-risk neuroblastoma. Pugh TJ*, Morozova O*, …, Gerhard DS, Hogarty MD, Jones SJM, Lander ES, Gabriel SB, Getz G, Seeger RC, Khan J, Marra MA, Meyerson M, Maris JM. Nature Genetics. 2013 Mar;45(3):279-84. PMID 23334666. (* contributed equally)
10. Medulloblastoma exome sequencing uncovers subtype-specific somatic mutations. Pugh TJ, …, Lichter P, Pfister SM, Roberts TM, Meyerson M, Pomeroy SL, Cho YJ. Nature. 2012 Aug 2;488(7409):106-10. PMID 22820256.

Functionalizing SWI/SNF chromatin remodeller mutations in rare and common tumours

Terry Fox New Investigator Operating Grant (2017-2020)

Investigator: Peter C. Stirling, BC Cancer

Mentoring Program: The Terry Fox New Frontiers Program Project Grant in the Genomics of Forme Fruste Tumours: New Vistas on Cancer Biology and Management

Scientific Summary: Genome sequencing has uncovered frequent somatic loss-of-function mutations in chromatin remodeling proteins in the switch/sucrose nonfermentable (SWI/SNF) complexes BAF and PBAF as underlying drivers of cancer formation. Mutations in these complexes shift the epigenetic landscape of tumour cells, changing gene expression in a cell context-specific manner, and affecting cancer cell survival, cell cycle progression, differentiation and DNA repair pathways. Moreover, SWI/SNF mutations are found in both rare tumours such as synovial sarcoma and malignant rhabdoid tumours, as well as more common ovarian, lung, breast and other cancers. At many genes, SWI/SNF works in opposition to another epigenetic modifier called the polycomb repressive complex 2 (PRC2). Upsetting the balance of SWI/SNF and PRC2 regulation is one of the ways in which these mutations may lead to cancer.

Recently, DNA: RNA hybrids forming in the genome were shown to occlude PRC2 binding and gene silencing. These structures, called R-loops, have normal functions in regulating gene expression, but also cause genome instability when they accumulate in excess or are dysregulated. R-loops at gene promoters were conversely shown to recruit a histone acetyltransferase, Tip60, which works downstream of SWI/SNF complexes in gene activation. These data suggest a crucial role for R-loops in regulating SWI/SNF targets, preventing PRC2 binding and promoting Tip60 binding.

During the tenure of this TFRI New Investigator award we have made considerable progress on our proposed plans to define a role for the SWI/SNF complex in R-loop associated genome instability and to create genome-wide networks of fitness genes in SWI/SNF deficient cancer cell models. This work has focused on ARID1A, which is the most frequently mutated subunit of human SWI/SNF complexes across cancer types. We have found that loss of ARID1A leads to increased DNA damage and replication stress in a manner that is dependent on R-loop accumulation. This effect is mediated in part by altered recruitment of the cancer drug target TOP2A to chromatin. We have also deployed genome-wide CRISPR drop out screens in cells that lack ARID1A. These screens reveal that DNA damage checkpoint and other factors become essential for cell survival when ARID1A is deficient. These data coalesce into a picture where new targets for selective killing of ARID1A-deficient tumours are to be found in the replication and DNA damaging signaling space. We are moving forward with validating such targets genetically in a variety of models with a view to future translational studies.

List of Key Publications:

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2. Tam AS & Stirling PC. Splicing, genome stability and disease: splice like your genome depends on it! *Curr Genet* 65: 905-912, 2019. http://www.ncbi.nlm.nih.gov/pubmed/30953124 (NO)
3. Tam AS, Sihota TS, Milbury KL, Zhang A, Mathew V & Stirling PC. Selective defects in gene expression control genome instability in yeast splicing mutants. *Mol Biol Cell* 30: 191-200, 201 http://www.ncbi.nlm.nih.gov/pubmed/30462576 (NO)
4. Parameswaran S, Vizeacoumar FS, Kalyanasundaram Bhanumathy K, Qin F, Islam MF, Toosi BM, Cunningham CE, Mousseau DD, Uppalapati MC, Stirling PC, Wu Y, Bonham K, Freywald A, Li H & Vizeacoumar FJ. Molecular characterization of an MLL1 fusion and its role in chromosomal instability. *Mol Oncol* 13: 422-440, 2019. http://www.ncbi.nlm.nih.gov/pubmed/30548174
5. Milbury KL, Paul B, Lari A, Fowler C, Montpetit B & Stirling PC. Exonuclease domain mutants of yeast DIS3 display genome instability. *Nucleus* 10: 21-32, 2019. http://www.ncbi.nlm.nih.gov/pubmed/30724665
6. Chang EY, Tsai S, Aristizabal MJ, Wells JP, Coulombe Y, Busatto FF, Chang YA, Kumar A, Dan Zhu Y, Wang AY, Fournier LA, Hieter P, Kobor MS, Masson JY & Stirling PC. MRE11-RAD50-NSB1 promotes Fanconi. Anemia R-loop suppression at transcription-replication conflicts. *Nat Commun* 10: 4265, 2019.
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8. Segovia R, Shen Y, Lujan SA, Jones SJ & Stirling PC. Hypermutation signature reveals a slippage and realignment model of translesion synthesis by Rev3 polymerase in cisplatin-treated yeast. *Proc Natl Acad Sci USA* 114: 2553-2668, 2017.
9. Xu H, Di Antonio M, McKinney S, Mathew V, Ho B, O'Neil NJ, Santos ND, Silvester J, Wei V, Garcia J, Kabeer F, Lai D, Soriano P, Banáth J, Chiu DS, Yap D, Le DD, Ye FB, Zhang A, Thu K, Soong J, Lin SC, Tsai AH, Osako T, Algara T, Saunders DN, Wong J, Xian J, Bally MB, Brenton JD, Brown GW, Shah SP, Cescon D, Mak TW, Caldas C, Stirling PC, Hieter P, Balasubramanian S & Aparicio SA. CX-5461 is a DNA G-quadruplex stabilizer with selective lethality in BRCA1/2 deficient tumours. *Nat Commun* 8: 14432, 2017.
10. Chang EY & Stirling PC. Replication Fork Protection Factors Controlling R-Loop Bypass and Suppression. *Genes* (Basel) 8: 1-11, 2017. (Review)
11. Stirling PC & Hieter P. Canonical DNA repair pathways influence R-loop driven genome instability. *J Mol Biol,* 2017 (Epub ahead of print). (Review)
12. Novoa C, Ang JS, & Stirling PC. The A-Like Faker Assay for Measuring Yeast Chromosome III Stability. Methods *Mol Biol*. In Press.
13. Segovia R, Mathew V, Tam AS, & Stirling PC. Genome-wide bisulfite sensitivity profiling of yeast suggests bisulfite inhibits transcription. *Mutat Res*. 821: 14-19, 2017.
14. Stirling PC, Shen Y, Corbett R, Jones SJ, Hieter P. Genome destabilizing mutator alleles drive specific mutational trajectories in Saccharomyces cerevisiae. *Genetics* 196: 403-412, 2014.
15. van Pel DM, Stirling PC, Minaker SW, Sipahimalani P, Hieter P. 2013. Saccharomyces cerevisiae genetics predicts candidate therapeutic genetic interactions at the mammalian replication fork. *G3* (Bethesda, Md) 3: 273-282, 2013
16. Stirling PC, Chan YA, Minaker SW, Aristizabal MJ, Barrett I, Sipahimalani P, Kobor MS, Hieter P. R-loop-mediated genome instability in mRNA cleavage and polyadenylation mutants. *Genes & development* 26: 163-175, 2012.

Next generation cancer immunovirotherapy: Heterologous oncolytic prime-boost enhanced with selected immunomodulators

Terry Fox New Investigator Operating Grant (2016-2018)

Investigator: Guy Ungerechts, Ottawa Hospital Research Institute

Mentoring Program: The Terry Fox New Frontiers Program Project Grant in Canadian Oncolytic Virus Consortium (COVCo)

Oncolytic viruses (OVs) were originally designed to selectively replicate in and destroy cancer cells. Recently, it has become increasingly appreciated that oncolytic virotherapy is also a potent stimulator of anti-tumor immune responses. Different OVs have been shown to induce immunogenic cell death, resulting in effective cross-presentation of tumor-associated antigens (TAA) to T-cells. In previous studies, we have developed strategies to further enhance these effects by engineering OVs for targeted immunomodulation.

Measles viruses (MeVs) derived from a vaccine strain are a flexible platform to develop safe and effective oncolytic vectors. Previously, we have demonstrated that various immunomodulators, like cytokines (GM CSF and IL-12), bispecific T-cell engagers (BiTEs) and immune checkpoint inhibitors (antibodies against CTLA 4 and PD L1), can improve MeV therapy in animal tumour models. Currently, several clinical trials with MeV for treatment of different tumour entities are ongoing.

The Terry Fox Canadian Oncolytic Virus Consortium (COVCo) is a leader in the development of oncolytic therapies. The COVCo has pioneered a prime-boost approach using a vaccine vector and oncolytic Maraba virus which is now under clinical investigation. Within this program, we modify this approach to increase its therapeutic potential. Instead of a non-replicating priming vector, we will generate replicating oncolytic MeV vectors encoding tumour antigens. Using established preclinical immunocompetent models, we assess therapeutic effects of measles virus vaccination as single agent as well as in combination with a heterologous Maraba virus boost. We investigate the mode of action by characterizing the immunological tumour environment after therapy. For synergistic anti-cancer activity, we combine this approach with vector-mediated immunomodulators. Further combination approaches with immune checkpoint inhibitors are established to overcome tumour immune evasion.

Specific Aims:

1. Development and proof-of-concept: Directing the immune response to tumour-specific antigens using oncolytic measles virus with or without Maraba virus as a boosting vector. We generate oncolytic MeV encoding selected tumour antigens, like the melanoma-associated antigens dopachrome tautomerase (DCT) and MAGE-A3 to complement the existing Maraba-MAGE-A3 concept developed by the COVCo. We investigate therapeutic effects and mechanism of action in preclinical models of malignant melanoma. Proof-of-concept and mechanistic studies are carried out in the established B16 murine melanoma model using DCT as an exemplary tumor antigen. 2. Combination therapies: Enhancement of TAA-directed immune responses by combination of the MeV/Maraba-DCT prime-boost with IL-12, BiTEs and immune checkpoint inhibitors. 3. Translational approach: Heterologous oncolytic vaccination using a MeV prime / Maraba-MAGE-A3 boost for future clinical application. We investigate therapeutic effects and mechanism of action in preclinical models of malignant melanoma. Enhancement of efficacy by combination of the MeV/Maraba-MAGE-A3 prime-boost with IL-12, BiTEs and immune checkpoint inhibitors will be evaluated in the established B16 murine model.

Updates:

1. Viral vector construction: Using our MeV vector platform, MeV encoding the DCT tumour antigen was generated as well as MeV encoding the green fluorescent protein and the parental "MeV-empty" in parallel as controls. These recombinant viruses were efficiently rescued, amplified and purified, leading to titers in the same range. 2. Viral replication and oncolytic activity: Replication kinetics and oncolytic properties were compared to parental MeV *in vitro*. After infection of tumour cells and measurement of progeny particle production, we confirmed that MeV-DCT and the control MeV-empty are able to efficiently transduce and replicate in these cells. Through selected targeting, toxicity and cell lysis was specifically achieved in the B16 tumour cell line. 3. Virus-mediated TAA expression: Expression of the MeV-encoded DCT in infected B16 tumour cells was confirmed by RT-PCR and Western Blot. Tumour cells of control treatments showed a basal level of endogenous DCT which is clearly enhanced in the MeV-DCT infected sample. 4. MeV-DCT induces specific anti-DCT T-cell activation: The tumour antigen-specific activation of immune effector cells was assessed *in vitro* by using a DCT-specific murine T-cell clone incubated with different stimuli. To show the capability of MeV-DCT to activate DCT-specific T-cells, MC38 colon carcinoma cells without endogenous DCT expression were infected with either MeV-DCT or MeV-empty and subsequently incubated with the T-cell clone. The Interferon (IFN)-γ ELISpot assay showed a specific and pronounced activation of anti-DCT T cells by MeV-DCT infected tumour cells compared to the controls. This confirms that MeV expressing a tumour-associated antigen can efficiently mediate the stimulation of tumour antigen-specific T-cells. This positive result is the fundamental proof-of-concept of our oncolytic prime-boost approach *in vitro*. 5. Prime-boost tumour vaccination in mice: The *in vivo* study to assess the oncolytic prime-boost concept to elicit a tumour antigen-specific immune response is ongoing.

List of Key Publications:

1. Veinalde R, Grossardt C, Hartmann L, Bourgeois-Daigneault MC, Bell JC, Jäger D, von Kalle C, Ungerechts G, Engeland CE. Oncolytic measles virus encoding interleukin-12 mediates potent antitumor effects through T cell activation. Oncoimmunology. 2017 Jan 31;6(4):e1285992.

The Terry Fox New Frontiers Program Project Grant: Canadian Oncolytic Virus Consortium (COVCo)

(2017-2022)

Project Leader: John Bell, Ottawa Hospital Research Institute (OHRI)

Investigators: Harold Atkins, Rebecca Auer, Jean-Simon Diallo, Guy Ungerechts, OHRI; David Stojdl, Tommy Alain, Children's Hospital of Eastern Ontario; Brad Nelson, Deeley Research Institute/BC Cancer Agency; Nahum Sonenberg, McGill University; Brian Lichty, Jonathan Bramson, Yonghong Wan, Karen Mossman, McMaster University; Andrea McCart, UHN; Byram Bridle, University of Guelph.

Scientific Summary: Our program is directed toward the discovery and testing of novel replicating anti-cancer viruses, and complimentary immunotherapeutic strategies for the treatment of cancer. We believe that for most oncolytic virus (OV) platforms, infection and oncolysis are essential for acute tumour cell killing, but long-term benefit in most patients is due to the generation of anti-tumour immune responses. We predict that there are multiple steps during OV-mediated therapy that can be manipulated to improve therapeutic outcomes. COVCo conducts inter-related research projects that are designed to address different aspects of oncolytic virus therapy: augmenting the initial tumour cell infection event, use of OVs as vehicles to deliver cargo to manipulate the tumour microenvironment, enhancement of the inherent vaccination mechanism-of-action of the OV platform, and coupling OV therapy with other immune-based therapeutic modalities, particularly those which elicit a personalized anti-tumour response. The successful execution of this program will lead to the development of novel therapeutic strategies that we will work with partners to accelerate into the clinic.

Program Objectives & Ongoing Studies: Our goal is to stay at the leading edge of OV discovery/development and advance rational combinations of therapeutic strategies. Through our research we will: 1. Develop optimal genetic and chemical strategies for enhancing OV spread within tumours; 2. Attack the tumour micro-environment using innovative therapeutic combination strategies; and; 3. Understand and exploit OV interactions with the adaptive and innate immune systems.

Highlights: COVCo's collaborative team has been effective in creating innovative therapeutic strategies and rapidly translating them into clinical testing. In the following are examples of some of the projects and productivity that have emerged from the COVCo Program:

- Oncolytic Vaccinia Virus: COVCo supported the advancement of a vaccinia virus candidate into clinical testing. This virus (Pexa-Vec) has advanced into Phase III testing (NCT02562755) for the treatment of liver cancer and is being developed by a partnership between SillaJen Biotherapeutics and Transgene Inc. COVCo discoveries provided the underpinnings of the mechanism of action of this product and many of the manufacturing processes and assays used for its clinical advancement. All of the virus product used for 11 worldwide trials leading up to this pivotal Phase III study were manufactured at the OHRI.
- Oncolytic Virus Vaccine (OVV) Platform: This COVCo-developed technology has advanced into clinical testing (NCT02285816) and has spawned a Canadian based Biotech company, Turnstone Biologics. In 2017, a second trial combining OVV technology with pembrolizumab has been initiated (NCT02879760), with a third trial directed at cancers caused by HPV infection is being initiated by the end of 2017.
- Viral Sensitizer (VSe) technology: There has been great interest from Pharma on the application of VSe compounds for use in infectious disease vaccine manufacturing, but within the COVCo team VSe technology is being advanced for cancer applications.
- T Cell Antigen Coupler Technology: Triumvira Immunologics is a new Canadian Biotech company built on technology developed within COVCo, advancing early-stage clinical development of an antigen-specific novel engineered T cell receptor complex.
- Manuscripts and Patents: Over the last five years of funding, COVCo has cited Terry Fox support on 43 publications and filed 36 patent applications, the vast majority of these are co-authored by multiple COVCo investigators.

List of Key Publications:

1. Ilkow CS, Marguerie M, Batenchuk C, Mayer J, Ben Neriah D, Cousineau S, Falls T, Jennings V, Boileau M, Bellamy D, Bastin D, Tanese de Souza C, Alkayyal A, Zhang J, Le Boeuf F, Arulanandam R, Stubbert L, Sampath P, Thorne S, Paramanthan P, Chatterjee A, Strieter RM, Burdick M, Addison C, Stojdl DF, Atkins HL, Auer R, Diallo JS, Lichty B, and Bell JC. Reciprocal cellular cross-talk within the tumour microenvironment promotes oncolytic virus activity. *Nat Med.* 2015 May; 21(5):530-6. PMID:25894825
2. Arulanandam R, Batenchuk C, Angarita FA, Ottolino-Perry K, Cousineau S, Mottashed A, Burgess E, Falls TJ, De Silva N, Tsang J, Howe GA, Bourgeois-Daigneault MC, Conrad DP, Daneshmand M, Breitbach CJ, Kirn DH, Raptis L, Sad S, Atkins H, Huh MS, Diallo JS, Lichty B, Ilkow CS, Le Boeuf F, Addison CL, McCart JA and Bell JC. VEGF-mediated induction of PRD1-BF1/Blimp1 expression sensitizes tumour vasculature to oncolytic virus infection. *Cancer Cell.* 2015 Jul 22 pii: S1535-6108(15)00219-6. PMID:26212250
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7. Wang F, Alain T, Szretter KJ, Stephenson K, Pol JG, Atherton MJ, Hoang HD, Fonseca BD, Zakaria C, Chen L, Rangwala Z, Hesch A, Chan ESY, Tuinman C, Suthar MS, Jiang Z, Ashkar AA, Thomas G, Kozma SC, Gale M Jr, Fitzgerald KA, Diamond MS, Mossman K, Sonenberg N, Wan Y, Lichty BD. S6K-STING interaction regulates cytosolic DNA-mediated activation of the transcription factor IRF3. *Nat Immunol.* 2016 May; 17(5):514-522. PMID:27043414

The Terry Fox New Frontiers Program Project Grant in ultrasound and MRI for cancer therapy

(2016-2021)

Investigators: Gregory J. Czarnota[1], Kullervo Hynynen[1], Gregory J. Stanisz[1], Michael C. Kolios[2], Alex Kiss[1], Ali Sadeghi-Naini[1], William Tran[1]. [1] Sunnybrook Research Institute, Toronto, ON. [2] Ryerson University, Toronto, ON

Scientific Summary: Radiation and chemotherapy remain foundational elements of care for cancer patients. Our goal is to make these techniques, two of the most common cancer treatments, significantly better through personalized cancer care. We are developing new and innovative ultrasound and MRI imaging methods to detect whether a treatment is working earlier in the treatment process than ever. We are also developing new image-guided treatments using ultrasound and MRI to make chemotherapy and radiation therapy more effective and with fewer side effects, ultimately leading to better patient quality of life.

Our translational research at the Odette Cancer Centre at Sunnybrook Health Sciences Centre/University of Toronto is focused on breast cancer. We are using ultrasound and MRI to improve chemotherapy and radiation treatments in four interrelated strategic areas: (1) ultrasound therapy for radiation enhancement, (2) ultrasound-based chemotherapy enhancement with a focus on microbubble-mediated endothelial cell perturbation to enhance tumour response, (3) quantitative ultrasound for therapy monitoring, and (4) novel methods in metabolic MRI imaging. This research spans new innovative cancer therapies, new therapy-response assessment methods, and the first-in-human treatment of cancer using the methods developed here.

We are developing novel ultrasound-based methods for enhancing therapy, whereby ultrasound-stimulated and microbubble-mediated endothelial cell perturbation can significantly enhance the effectiveness of radiation and chemotherapy in several ways. For radiation enhancement, we are optimizing this ASMase-activated, ceramide-dependent effect and scaling it up for world-first, proof-of-principle evaluation in cancer patients. For chemotherapy enhancement, we are developing bubble-tracking technology to facilitate vascular permeabilization with ultrasound. We are, in tandem, developing new state-of-the-art ultrasound therapy technology for the integration of these new methods with microbubble-based modulation on a clinical device. In order to monitor these treatments, we are further developing and adapting our quantitative ultrasound methods, developed for therapy response assessment. This work is being conducted using new textural-based methodologies applied to newer applications, such as *a priori* prediction of outcomes to chemotherapy and characterization of malignant tumours. We are also developing and evaluating new contrast-enhanced saturation transfer (CEST)-based MRI techniques to develop new metabolic-based imaging methods. These will be integrated into the planned treatment platform for ultrasound-based chemotherapy and radiation enhancement and will be used in cancer patients for therapy monitoring

Our proposed research will use ultrasound and MRI methods to enhance chemotherapy and radiation treatments. Novel therapy monitoring methods will be combined with new therapies, and together these technologies will disrupt and transform state-of-the art cancer treatment delivery, ultimately leading to improved care for cancer patients.

The Terry Fox New Frontiers Program Project Grant in the development of stemness-based prognostic biomarkers and therapeutic targets

(2015-2020)

Investigators: John Dick, Norman Iscove, and Rodger Tiedemann, Princess Margaret Cancer Centre, University Health Network; Gary Bader, University of Toronto; and Peter Dirks, The Hospital for Sick Children.

Scientific Summary: Tumour heterogeneity plays a major role in therapy failure resulting in disease progression and recurrence. Our team is guided by the central hypothesis that both sub-clonal genetic diversity and the existence of cellular hierarchies contributes to tumour heterogeneity and it is the unified effect of both that influences the stemness properties of individual tumour cells; ultimately, stemness is the key biological property that governs patient outcomes.

We are focused on defining the determinants of stemness for high-risk cancers including acute myeloid leukemia (AML), myeloma and glioblastomas multiforma (GBM) that have poor outcome and urgently require effective therapies. Our vision is translating the unique knowledge we have gained into a new generation of clinically relevant biomarkers and therapeutics that target the vulnerabilities of CSCs, reducing therapy failure and increasing patient survival.

Each project is built upon state-of-the-art CSCs assays, using either primary human cancer cells or engineered murine models, and novel approaches to screen for vulnerabilities of cancer cells in general, and CSCs specifically. We will also define the genetic basis for subclonal diversity in myeloma and GBM. Our focus is to deploy specific functional assays that measure self-renewal, the key hallmark of the stemness state, to gain mechanistic insight into how selected genetic, epigenetic or metabolic targets govern CSCs function for each cancer type.

The vast amount of data will be integrated into a single bioinformatic core to gain insight into stemness at a pathway level, which will be central to therapeutic target identification. The specific aims for each project coalesce around three major translational outcomes: the development of stemness-based biomarkers for predicting clinical features that will enable improved clinical cancer management; the identification of new therapeutic targets that ensure eradication of bulk tumour cells as well as the CSCs that lie at the root of the cancer; and pre-clinical development of targets using state-of-the-art primary cancer xenografts that our team pioneered.

Achievements: Expansion of our understanding of the molecular basis of sustained self-renewal is leading to identification of therapeutic targets with real potential for eradication of AML, GBM and myeloma. We have discovered several targets for stemness in AML, and GBM and we are evaluating their therapeutic effects and building the knowledge base for the linkage between targeting stemness and treating cancer effectively. We have developed novel methods to analyze single cell RNA-seq data in use by a wide range of cancer research projects and ways of integrating this data to establish focus for evaluation of targets.

List of Key Publications:

1. Kaufmann KB, Garcia-Prat L, Liu Q, Ng SWK, Takayanagi SI, Mitchell A, Wienholds E, van Galen P, Cumbaa CA, Tsay MJ, Pastrello C, Wagenblast E, Krivdova G, Minden MD, Lechman ER, Zandi S, Jurisica I, Wang JCY, Xie SZ, Dick JE. A stemness screen reveals C3orf54/INKA1 as a promoter of human leukemia stem cell latency. *Blood.* 2019 May 16;133(20):2198-2211. doi: 10.1182/blood-2018-10-881441. Epub 2019 Feb 22.
2. Xie SZ, Garcia-Prat L, Voisin V, Murison A, Ferrari R, Gan O, Takayama N, Kaufmann KB, Patel I, Lee E, Jargstorf J, Holmes G, Romm G, Zhou S, Boutzen H, Medina T, Vedi A, De Carvalho D, Luberto C, Bader GD, Laurenti E, Lupien M, and **Dick JE** Sphingolipid Modulation Activates Proteostasis Programs To Govern Human Hematopoietic Stem Cell Self-renewal (*Cell Stem Cell,* in press)
3. MacLeod G, Bozek DA, Rajakulendran N, Monteiro V, Ahmadi M, Steinhart Z, Kushida MM, Yu H, Coutinho FJ, Cavalli FMG, Restall I, Hao X, Hart T, Luchman HA, Weiss S, Dirks PB, Angers S. Cell Rep. 2019 Apr 16;27(3):971-986.e9. doi: 10.1016/j.celrep.2019.03.047.Genome-Wide CRISPR-Cas9 Screens Expose Genetic Vulnerabilities and Mechanisms of Temozolomide Sensitivity in Glioblastoma Stem Cells. *Cell* Rep 27, 971-986 e979, doi:10.1016/j.celrep.2019.03.047(2019)
4. Rajakulendran N, Rowland KJ, Selvadurai HJ, Ahmadi M, Park NI, Naumenko S, Dolma S, Ward RJ, So M, Lee L, MacLeod G, Pasiliao C, Brandon C, Clarke ID, Cusimano MD, Bernstein M, Batada N, Angers S, Dirks PB. Wnt and Notch signaling govern self-renewal and differentiation in a subset of human glioblastoma stem cells. *Genes* Dev. 2019 May 1;33(9-10):498-510. doi: 10.1101/gad.321968.118. PMID: 30842215
5. Park NI, Guilhamon P, Desai K, McAdam RF, Langille E, O'Connor M, Lan X, Whetstone H, Coutinho FJ, Vanner RJ, Ling E, Prinos P, Lee L, Selvadurai H, Atwal G, Kushida M, Clarke ID, Voisin V, Cusimano MD, Bernstein M, Das S, Bader G, Arrowsmith CH, Angers S, Huang X, Lupien M, Dirks PB. ASCL1 Reorganizes Chromatin to Direct Neuronal Fate and Suppress Tumorigenicity of Glioblastoma Stem Cells. *Cell Stem Cell* 21, 209-224 e207, doi:10.1016/j.stem.2017.06.004 (2017).
6. Cavalli FMG, Remke M, Rampasek L, Peacock J, Shih DJH, Luu B, Garzia L, et al 79 coauthors… Tabori U, Bouffet E, Bartels U, Dirks PB, Rutka JT, Bader GD, Reimand J, Goldenberg A, Ramaswamy V, Taylor MD.Intertumoral Heterogeneity within Medulloblastoma Subgroups. *Cancer Cell* 31, 737-754 e736, doi:10.1016/j.ccell.2017.05.005 (2017).
7. Morrissy AS, Cavalli FMG, Remke M, Ramaswamy V, Shih DJH, et al 58 coauthors Dirks P, Huang A, Bouffet E, Rutka JT, Bader GD, Swanton C, Ma Y, Moore RA, Mungall AJ, Majewski J, Jones SJM, Das S, Malkin D, Jabado N, Marra MA, Taylor MD. Spatial heterogeneity in medulloblastoma. *Nat Genet* 49, 780-788, doi:10.1038/ng.3838 (2017).

Manuscripts in Preparation

To be submitted Fall 2019: Takayama N*, Murison A*, Takayanagi SI, Arlidge C, Zhou S, García-Prat L, Chan-Seng-Yue M, Zandi S, Gan O, Boutzen H, Kaufmann KB, Trotman-Grant A, Schoof E, Kron K, Lee JJY, Medina T, De Carvalho D, Taylor MD, Minden MD, Xie SZ, John E. Dick† and Mathieu Lupien†. Reprogrammed CTCF binding at chromatin loops regulates stemness in hematopoietic stem cells *shared first authors, †shared senior authors

In preparation for submission December 2019: SZ Xie, K Kaufmann, O Gan, E Laurenti, SWK Ng, M Chan-Seng Yue, Q Liu, L Garcia-Prat, SI Takayanagi, M Kleinau, J Jargostf, G Holmes, C Luberto, L Obeid, Y Hannun, JCY Wang, MD Minden, and JE Dick. Sphingosine-1-Phosphate Receptor 3 governs myeloid lineage priming in human hematopoietic and leukemia stem cells.

In preparation for submission Fall 2019: Madipour A, Erdmann N, Leung-Hagesteijn C, Konda J, Tagoug I, Tiedemann RE. The effects of chromosomal copy number variations on transcriptional programs at single cell resolution in multiple myeloma.

In early preparation (2020): Tagoug I, Madipour A, Erdmann N, Konda J, Chattaragada M, Leung-Hagesteijn C, Tiedemann RE. The genomic landscape of multiple myeloma pre-plasma cells and their role in disease

The Terry Fox New Frontiers Program Project Grant in targeting the adaptive molecular landscape in castrate-resistant prostate cancer

(2016-2021)

Investigators: Martin Gleave, Ralph Buttyan, Peter Black, Artem Cherkasov, Colin Collins, Michael Cox, Mads Daugaard, Xuesen Dong, Ladan Fazli, Emma Guns, Chris Ong, Paul Rennie, Alan So, Yuzhuo Wang, Alex Wyatt, Amina Zoubeidi: VPC, UBC; Hongshen Ma: UBC; Kim Chi, Poul Sorensen: VPC & BC Cancer; Faraz Hach, Cenk Sahinalp: VPC, SFU; H. Hansen He: Princess Margaret Cancer Centre & University of Toronto; Michael Pollak, McGill University

Scientific Summary: Progression to castrate resistance following androgen ablation is the main obstacle to improving survival for men with advanced prostate cancer and the central focus of our Terry Fox New Frontiers Program Project Grant, comprised of a multidisciplinary team of 23 scientists and clinicians. Androgen ablation precipitates a cascade of changes in transcriptional and signalling networks that provide a selective survival and growth advantage for sub-populations of the tumour cells, thereby accelerating progression and rendering cells more resistant to therapy.

Objectives:

- Elucidate genomic, molecular and cellular mechanisms responsible for progression to castrate resistance.
- Use this information to develop new therapies aimed at biologically relevant and tumour-specific targets and pathways to delay progression of late-stage disease.
- Partner with national clinical trials networks and industry to accelerate bench-to-bedside translation of our discovery science.

Our program consists of six individual yet highly integrated research projects spanning the research spectrum from discovery to treatment science:

- Project 1 investigates how stress adaptor proteins (YB-1, CLU, Hsp27) activated by androgen receptor pathway inhibitors help re-program the translatome and metabolome to enable tumour cell adaptation, survival, and treatment resistance;
- Projects 2 and 3 investigate genomic (ERG) alterations and developmental signaling (GLI) pathways that facilitate androgen receptor reactivation after androgen receptor pathway inhibition;
- Projects 4 and 5 investigate mechanisms enabling emergence of non-androgen receptor-driven castrate-resistant prostate cancer and neuroendocrine prostate cancer;

All projects are supported by five cores (Advanced Genomics and Computer Science, Pathology & Molecular Imaging, Tumour Models, Therapeutics Development, Translational Trials), all uniquely managed as a collective within a single organization focused on understanding and controlling treatment resistance.

In summary, our Program Project Grant in Targeting the Adaptive Molecular Landscape in Castrate-Resistant Prostate Cancer is a major catalyst for translational research that has enabled us to have bring several new therapies from bench to bedside. The program will help further accelerate discovery and validation of novel cellular and molecular targets and uncover mechanisms for treatment resistance.

By pooling our talents and resources into a single co-operative effort, we maximize our potential for solving the problem of prostate cancer progression in the most efficient manner. This program is an ideal example of how team science enables the discovery of underlying mechanisms of prostate cancer progression, facilitates the development of new multimodality therapies, and accelerates translation of research into clinical practice. The many publications related to this program listed below are evidence of our team-based interactions and productivity.

List of Key Publications:

1. Annala et al. Treatment Outcomes and Tumour Loss of Heterozygosity in Germline DNA Repair-deficient Prostate Cancer. *Eur Urol.* 2017 Jul;72(1):34-42. PMID: 28259476
2. Bishop et al. The Master Neural Transcription Factor BRN2 is an Androgen Receptor Suppressed Driver of Neuroendocrine Differentiation in Prostate Cancer. *Cancer Discov.* 2017 Jan; 7(1):54-71. PMID: 27784708
3. Butler et al. Discovery and characterization of small molecules targeting the DNA-binding ETS domain of ERG in prostate cancer. *Oncotarget* 2017; 8:42438-42454. PMID: 28465491
4. Chedgy et al. Moving towards personalized care: liquid biopsy predicts response to cisplatin in an unusual case of BRCA2-null neuroendocrine prostate cancer. *Clinical Genitourinary Cancer.* 2016 Apr; 14(2):e233-6. PMID: 26797585.
5. Chi et al. A Phase 1 Dose-Escalation Study of Apatorsen (OGX-427), an Antisense Inhibitor Targeting Heat Shock Protein 27 (Hsp27), in Patients with Castration Resistant Prostate Cancer and Other Advanced Cancers. *Ann Oncol.* 2016 Jun; 27(6):1116-22. pii: mdw068. Epub 2016 Feb 18. PMID: 27022067
6. Coleman et al. Cellular androgen content influences enzalutamide agonism of F877L mutant androgen receptor. *Oncotarget.* 2016 Jun 28;7(26):40690-40703 PMID: 27276681
7. Crea et al. Integrated analysis of the prostate cancer small-nucleolar transcriptome reveals SNORA55 as a driver of prostate cancer progression. *Mol Oncol.* 2016 May;10(5):693-703 PMID: 26809501.
8. Donmez et al. Clonality Inference from Single Tumour Samples Using Low-Coverage Sequence Data. *J Comput Biol.* 2017 Jun;24(6):515-523. PMID: 28056180.
9. Kockan et al. SiNVICT: ultra-sensitive detection of single nucleotide variants and indels in circulating tumour DNA. *Bioinformatics.* 2017 Jan 1;33(1):26-34. PMID: 27531099.
10. Luk et al. BIRC6-targeting as potential therapy for advanced, enzalutamide-resistant prostate cancer. *Clin Cancer Res.* 2017 Mar 15;23(6):1542-1551. PMID: 27663589.
11. Mo et al. Stromal Gene Expression is Predictive for Metastatic Primary Prostate Cancer. *European Urology* 2017 Mar 19. pii: S0302-2838(17)30166-5. PMID: 28330676
12. Saranchova et al. Discovery of a Metastatic Immune Escape Mechanism Initiated by the Loss of Expression of the Tumour Biomarker Interleukin-33. *Sci Rep.* 2016 Sep 13; 6:30555. PMID: 27619158.
13. Tam et al. Androgen receptor transcriptionally regulates semaphorin 3C in a GATA2-dependent manner. *Oncotarget.* 2017 Feb 7;8(6):9617-9633. PubMed PMID: 28038451
14. Wyatt AW, et al. Concordance of Circulating Tumour DNA and Matched Metastatic Tissue Biopsy in Prostate Cancer. *JNCI* 109(12), in press. Epub 29 June 2017.

The Terry Fox New Frontiers Program Project Grant in the genomics of forme fruste tumours: new vistas on cancer biology and treatment

(2013-2018)

Investigators: David Huntsman, Samuel Aparicio, Alexandre Bouchard-Côté, Martin Hirst, Chenghan Lee, Gregg Morin, Torsten Nielsen, Sohrab Shah, Peter Stirling, Poul Sorensen, T. Michael Underhill, Stephen Yip, UBC

Scientific Summary: This PPG is an innovative approach to making clinically meaningful cancer discoveries. *Forme fruste* tumours are rare cancers that are clinically and pathologically homogenous, typically driven by a limited number of genetic events. This property makes them ideal for research, but equally important, these tumours are in critical need of improved diagnostics and treatments. Additionally, discoveries made from the study of *forme fruste* tumours often have broader clinical relevance. Our past research on *forme fruste* tumours, which began with support from the Terry Fox Research Institute in 2010, has led to the discovery of many key mutations that drive these cancers or are associated with these cancers. Now, we are focusing on non-mutational events within these cancers shape their development and how can this information be used to better understand cancer biology, and develop diagnostics and treatments.

We have developed five inter-related sub-projects and one core facility that will work together to study these cancers. All projects will study two *forme fruste* tumours to promote cohesiveness and connectivity: these are synovial sarcoma and clear cell ovarian carcinoma. In addition, some projects will study additional *forme fruste* tumours: Sertoli Leydig cell tumours in Project 1, Ewing sarcoma in Projects 2 and 4, adult granulosa cell tumours in Project 3, and dedifferentiated endometrial carcinomas in Projects 3 and 4. Each project will focus on non-mutational features that impacts the development and progression of cancer. These studies will be supported by the *forme fruste* model systems that our team has developed with past funding.

Objectives:

Project 1 will study how cell context contributes to the development of cancer. This project will explore how the tumour microenvironment can either support or inhibit the development of cancer, and how the individual elements in the microenvironment impact cancer. In addition, Project 1 will focus on defining the cell of origin for cancers, and try to better understand how the cell of origin for different cancers contribute to cancer development. Project 2 will explore how adaptations to stress, such as the formation of stress granules, reflected in the set of expressed proteins – the translatome - contributes to neoplastic behaviour. This project will study the alterations to the translatome that occur under different stress conditions and how stress granules regulate tumour fitness and metastatic capacity. Further, this project will investigate if the stress adaptation that exists in cancers can lead to identification of vulnerabilities that provide therapeutic opportunities. Project 3 will survey the changes in the cancer transcription landscape that result from epigenomic perturbations and also identify gene targets and cellular potential that could be therapeutic targets. Project 4 will describe the clonal evolution that occurs in forme fruste tumours over time and in response to treatment, and use this to predict the behaviour tumour behaviour. In addition, this project will also identify the molecular determinants of clonal fitness in tumours. Project 5 will access the data generated by the other projects to develop algorithms that integrate cell context, stress adaptations, epigenomic alterations, and clonal fitness, to predict tumour behaviour and evolution, over time and in response to treatment. This will be done using multimodal single cell analysis.

These five sub-projects will be supported by a Translational Core that will validate the discoveries from the projects in human tissues. As these are rare tumours, sourcing a sufficient number of human samples for validation is not trivial and so this Core will rely on strong collaborations with researchers to maximize access to tissues. This Core will also translate findings from the Projects into diagnostic tools and validate and extend therapeutic discoveries from the Projects.

In addition to Core support, Projects and Cores will all work on development of methods and resources that can benefit all research in the program, such as the development of animal model systems, proteomic and epigenomic methods, single cell analysis methods, and therapeutic screens. The work described in this program will help improve the management and treatment for forme fruste tumours and provide general insights into the biology of cancer.

List of Key Publications:

1. Mazloomian, A., S. Araki, M. Ohori, A. M. El-Naggar, D. Yap, A. Bashashati, S. Nakao, P. H. Sorensen, A. Nakanishi, S. Shah and S. Aparicio. Pharmacological systems analysis defines EIF4A3 functions in cell-cycle and RNA stress granule formation. *Commun Biol* 2019; 2: 165; PMID: 31069274
2. Yu, J. S. E., S. Colborne, C. S. Hughes, G. B. Morin and T. O. Nielsen. The FUS-DDIT3 Interactome in Myxoid Liposarcoma. *Neoplasia* 2019; 21: 740-751; PMID: 31220736
3. Zhang, A. W., C. O'Flanagan, E. A. Chavez, J. L. P. Lim, N. Ceglia, A. McPherson, M. Wiens, P. Walters, T. Chan, B. Hewitson, D. Lai, A. Mottok, C. Sarkozy, L. Chong, T. Aoki, X. Wang, A. P. Weng, J. N. McAlpine, S. Aparicio, C. Steidl, K. R. Campbell and S. P. Shah. Probabilistic cell-type assignment of single-cell RNA-seq for tumor microenvironment profiling. *Nat Methods* 2019; 16: 1007-1015; PMID: 31501550
4. Campbell, K. R., A. Steif, E. Laks, H. Zahn, D. Lai, A. McPherson, H. Farahani, F. Kabeer, C. O'Flanagan, J. Biele, J. Brimhall, B. Wang, P. Walters, I. Consortium, A. Bouchard-Cote, S. Aparicio and S. P. Shah. clonealign: statistical integration of independent single-cell RNA and DNA sequencing data from human cancers. *Genome Biol* 2019; 20: 54; PMID: 30866997
5. Zhang, A. W., A. McPherson, K. Milne, D. R. Kroeger, P. T. Hamilton, A. Miranda, T. Funnell, N. Little, C. P. E. de Souza, S. Laan, S. LeDoux, D. R. Cochrane, J. L. P. Lim, W. Yang, A. Roth, M. A. Smith, J. Ho, K. Tse, T. Zeng, I. Shlafman, M. R. Mayo, R. Moore, H. Failmezger, A. Heindl, Y. K. Wang, A. Bashashati, D. S. Grewal, S. D. Brown, D. Lai, A. N. C. Wan, C. B. Nielsen, C. Huebner, B. Tessier-Cloutier, M. S. Anglesio, A. Bouchard-Cote, Y. Yuan, W. W. Wasserman, C. B. Gilks, A. N. Karnezis, S. Aparicio, J. N. McAlpine, D. G. Huntsman, R. A. Holt, B. H. Nelson and S. P. Shah. Interfaces of Malignant and Immunologic Clonal Dynamics in Ovarian Cancer. *Cell* 2018; 173: 1755-1769 e1722; PMID: 29754820
6. Hughes, C. S., P. H. Sorensen and G. B. Morin. A Standardized and Reproducible Proteomics Protocol for Bottom-Up Quantitative Analysis of Protein Samples Using SP3 and Mass Spectrometry. *Methods Mol Biol* 2019; 1959: 65-87; PMID: 30852816
7. Patel, N., J. Wang, K. Shiozawa, K. B. Jones, Y. Zhang, J. W. Prokop, G. G. Davenport, N. T. Nihira, Z. Hao, D. Wong, L. Brandsmeier, S. K. Meadows, A. V. Sampaio, R. V. Werff, M. Endo, M. R. Capecchi, K. M. McNagny, T. W. Mak, T. O. Nielsen, T. M. Underhill, R. M. Myers, T. Kondo and L. Su. HDAC2 Regulates Site-Specific Acetylation of MDM2 and Its Ubiquitination Signaling in Tumor Suppression. *iScience* 2019; 13: 43-54; PMID: 30818224
8. Banito, A., X. Li, A. N. Laporte, J. S. Roe, F. Sanchez-Vega, C. H. Huang, A. R. Dancsok, K. Hatzi, C. C. Chen, D. F. Tschaharganeh, R. Chandwani, N. Tasdemir, K. B. Jones, M. R. Capecchi, C. R. Vakoc, N. Schultz, M. Ladanyi, T. O. Nielsen and S. W. Lowe. The SS18-SSX Oncoprotein Hijacks KDM2B-PRC1.1 to Drive Synovial Sarcoma. *Cancer Cell* 2018; 33: 527-541 e528; PMID: 29502955

The Terry Fox New Frontiers Program Project Grant in discovery and therapeutic development of antibody-based targets in oncology

(2015-2019)

Investigators: Steven Jones (BC Cancer), Peter Bergqvist (adMare BioInnovations), Jahanshah Ashkani, François Bénard, Julie Rousseau, Gregg Morin (BC Cancer), Paul Schaffer (TRIUMF), Tomas Hudlicky (Brock University)

Scientific Summary: This proposal brings together a multidisciplinary team of experts from academia and industry focused on developing therapeutic antibody-based diagnostics, theranostics and therapeutics for newly discovered tumour-associated targets. It stands to bring together world-class genomics, bioinformatics, and proteomics capabilities (Genome Sciences Centre (GSC)) with both an established and a novel antibody-drug conjugate platform (adMare BioInnovations) and a clinically integrated imaging platform (BC Cancer). Underlying all of these individual programs is the cutting-edge antibody generation platform established at adMare BioInnovations which will generate panels of antibodies and derivatives to validate the targets identified from the bioinformatics program, and become the basis of therapeutics for the antibody-drug conjugate program and companion theranostics for the imaging program. Together this team will generate a suite of fully validated antibody-based therapeutics with matched theranostics which could be used individually or combined for next-generation targeted therapy.

List of Key Publications:

1. Beck A, Wurch T, Bailly C, Corvaia N. 2010. Strategies and challenges for the next generation of therapeutic antibodies. *Nat Rev Immunol* 10(5): 345-352.
2. Carter PJ, Senter PD. 2008. Antibody-drug conjugates for cancer therapy. *Cancer J* 14(3): 154-169.
3. Dargahi D, Swayze RD, Yee L, Bergqvist PJ, Hedberg BJ, Heravi-Moussavi A, Dullaghan EM, Dercho R, An J, Babcook JS, Jones SJ. 2014. A pan-cancer analysis of alternative splicing events reveals novel tumour-associated splice variants of matriptase. *Cancer Inform*. 13: 167-177.
4. Hughes B. 2010. Antibody-drug conjugates for cancer: poised to deliver? *Nat Rev Drug Discov* 9(9): 665-667.
5. Nelson AL, Dhimolea E, Reichert JM. 2010. Development trends for human monoclonal antibody therapeutics. *Nat Rev Drug Discov* 9(10): 767-774.
6. Webb S. 2011. Pharma interest surges in antibody-drug conjugates. *Nat Biotechnol* 29(4): 297-298

The Terry Fox New Frontiers Program Project Grant in exploiting pathogenic mechanisms in acute leukemia

(2017-2022)

Investigators: Aly Karsan, Connie Eaves, Andrew Weng, Martin Hirst, R. Keith Humphries, Peter Lansdorp, Gregg Morin, and Raewyn Broady, BC Cancer

Scientific Summary: Acute leukemia refers to a heterogeneous group of aggressive malignancies of the hematopoietic system with outcomes that have remained poor for decades. Further, the intensive chemotherapy regimens required for cure shortens and compromises quality of life. There is an urgent need to identify and target key dependencies of leukemic cells and contrast these with the requirements of normal cells in order to improve survival and quality of life. A major role in leukemia pathogenesis is played by perturbations in the regulation of the epigenome, and our team began five years ago to examine the relevance of such changes to the establishment and maintenance of leukemic cell populations. Our ongoing thrust now includes an expanding emphasis on creating de novo models of human as well as mouse leukemias, and on defining molecular mechanisms derived from the use of proteomics, RNAi and drug screens, and harnessing various single cell analytic techniques to model the critical alterations that cause this diverse group of diseases.

Overall Goal: The goal remains focused on developing the knowledge required to make improvements in the long-term outcomes of patients with leukemia, through the development of models, tools and derived information that will generate more effective and specific therapeutic strategies. We propose an integrated and synergistic program that will: elucidate the molecular dimensionality and regulation of normal CD34+ hematopoietic cell states (Project 1); characterize the impact of Skp1/Cul1/Fbxo11 (SCF) ubiquitination complex disruption on the propagation of AML (Project 2); define genetic, epigenetic and context-dependent growth mechanisms that reprogram normal human hematopoietic cells into malignant T-cells (Project 3); and evaluate novel clinically deliverable analogues of vitamin C through its role in reversing methylation gains and promoting differentiation in TET/IDH mutant AML (Project 4). Two Cores will provide state-of-the-art, program-wide access to primary human cells, associated patient data, novel immunodeficient mouse strains and genomic, epigenomic, mass cytometric and bioinformatic tools as well as continued development of these assets.

Expected Outcomes and Impact of Research: Our program is designed to identify and dissect novel vulnerabilities of human leukemic cells in a detailed comparison with processes required to support normal hematopoietic cell development. We will reveal molecular mechanisms that underpin growth, survival, and self-renewal in human leukemias in order to devise therapeutic strategies that yield more durable remissions and yet limit toxicities associated with current therapies. Partnerships with clinical groups and the Centre for Drug Research and Development will enable translation of these findings first in preclinical models and ultimately in clinical applications.

List of Key Publications:

1. Balani S, Nguyen LV & Eaves CJ. Modeling the process of human tumourigenesis. Nat Commun 8: 15422, 2017. PMID: 28541307.
2. Knapp DJ, Hammond CA, Aghaeepour N, Miller PH, Pellacani D, Beer PA, Sachs K, Qiao W, Wang W, Humphries RK, Sauvageau G, Zandstra PW, Bendall SC, Nolan GP, Hansen C, Eaves CJ. Distinct signaling programs control human hematopoietic stem cell survival and proliferation. Blood 129: 307-318, 2017. PMID: 27827829.
3. Knapp DJHF, Hammond CA, Miller PH, Rabu GM, Beer PA, Ricicova M, Lecault V, Da Costa D, VanInsberghe M, Cheung AM, Pellacani D, Piret J, Hansen C & Eaves CJ. Dissociation of survival, proliferation and state control in human hematopoietic stem cells. Stem Cell Reports 8:152-162, 2017. PMID: 28076756.
4. Miller PH, Rabu G, MacAldaz M, Knapp DJ, Cheung AM, Dhillon K, Nakamichi N, Beer PA, Shultz LD, Humphries RK & Eaves CJ. Analysis of parameters that affect human hematopoietic cell outputs in mutant c-kit-immunodeficient mice. Exp Hematol 48: 41-49, 2017. PMID: 28087429.
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6. Maetzig T, Ruschmann J, Lai CK, Ngom M, Imren S, Rosten P, Norddahl GL, von Krosigk N, Milde LS, May C, Selich A, Rothe M, Dhillon I, Schambach A & Humphries RK. A lentiviral fluorescent genetic barcoding system for flow cytometry-based multiplex tracking. Mol Ther 25: 606-620, 2017. PMID: 28253481.
7. O'Neill K, Hills M, Gottlieb M, Karsan A & Lansdorp P. Assembling Draft Genomes using contiBAIT. Bioinformatics 33: 2737-2739, 2017. PMID: 28475666.
8. Giambra V, Gusscott S, Gracias D, Song R, Lam SH, Panelli P, Tyshchenko K, Jenkins CE, Hoofd C, Lorzadeh A, Carles S, Hirst M, Eaves C & Weng AP. Epigenetic restoration of fetal-like IGF1 signaling inhibits leukemia stem cell activity. Cell Stem Cell 23:714-726, 2018. PMID: 30269902.
9. Mingay M, Chaturvedi A, Bilenky M, Cao Q, Jackson L, Hui T, Moksa M, Heravi-Moussavi A, Humphries RK, Heuser M & Hirst M. Vitamin C-induced epigenomic remodelling in IDH1 mutant acute myeloid leukaemia. Leukemia 32: 11-20, 2018. PMID: 28663574.
10. Hui T, Cao Q, Wegrzyn-Woltosz J, O'Neill K, Hammond CA, Knapp DJHF, Laks E, Moksa M, Aparicio S, Eaves CJ, Karsan A, Hirst M. High-resolution single-cell DNA methylation measurements reveal epigenetically distinct hematopoietic stem cell subpopulations. Stem Cell Reports 11: 578-592, 2018. PMID: 30078558.
11. Jenkins CE*, Gusscott S*, Wong RJ, Shevchuk OO, Rana G, Giambra V, Tyshchenko K, Islam R, Hirst M & Weng AP. RUNX1promotes cell growth in human T-cell acute lymphoblastic leukemia by transcriptional regulation of key target genes. Experimental Hematol 64: 84-96, 2018. (*co-first authors). PMID: 29733873.
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The Terry Fox New Frontiers Program Project Grant in triggers and targets in the tumour microenvironment: hypoxia and beyond

(2019-2025)

Investigators: Marianne Koritzinsky, Michael Milosevic, Alejandro Berlin, David Brooks, Hansen He, Tracey McGaha, Bradly Wouters, Gelareh Zadeh, Princess Margaret Cancer Centre, Toronto, Canada

Scientific Summary: Solid tumours establish an ecosystem of cancer cells, vasculature, extracellular matrix and immune cell infiltrates that exist along oxygen and nutrient gradients. The cellular responses and interactions within this complex tumor microenvironment contribute to aggressive cancer growth, immune evasion and treatment resistance. As a team, our main objective is to elucidate mechanisms triggered by the tumour microenvironment that drive aggressive cancer phenotypes and treatment resistance, and to target these mechanisms for improved cancer outcomes. Our focus is on tumour histologies and sites with strong clinical evidence implicating hypoxia or other aspects of the abnormal tumour microenvironment as a driver of poor outcomes, including glioblastoma multiforme (GBM), pancreatic cancer, prostate cancer and cervical cancer.

Objectives: Building on our previous successes bridging the divide between the laboratory and the clinic, the Program includes four integrated projects each with strong translational elements and led jointly by a basic scientist and a clinician scientist.

The objective of **Project 1** (Koritzinsky and McGaha) is to elucidate how cancer cells and stromal compartments interact in pancreatic cancer to maintain aggressive tumour growth and treatment resistance. We showed previously that macrophage polarization strongly influences the pancreatic tumour microenvironment and, in turn, tumour growth and treatment response. We will build on these findings to identify hypoxia-dependent signaling mechanisms that govern cancer cell secretion and macrophage polarization, and determine how these signaling mechanisms influence vascularization, oxygenation and immune infiltrates in the tumour microenvironment. We will target these signaling pathways using genetic and pharmacological approaches in experimental tumour models, with a focus on treatments that can readily be translated to the clinic.

Project 2 (Wouters and Zadeh) will investigate hypoxia, angiogenesis and immune infiltrates in GBM. Our recent work has shown that hypoxic GBM tumours exhibit a hypomethylated DNA signature, and that hypoxia-induced modifications of the epigenome can drive cancer stemness. We will further explore the hypothesis that hypoxia is associated with distinct epigenetic alterations in GBM that drive tolerance to the harsh microenvironment, promote stemness and resistance to therapy, and can be exploited for therapeutic benefit. Key epigenetic drivers will be targeted in orthotopic patient-derived xenografts using an existing drug library.

Project 3 (Berlin and He) will focus on the interplay between hypoxia, epigenetics and differentiation in castrate-resistant prostate cancer (CRPC). Trans-differentiation of prostate adenocarcinomas to neuroendocrine (NE) histology confers aggressive behaviour and treatment resistance. Our team has previously reported that hypoxia synergizes with master transcriptional regulators of prostate cancer trans-differentiation. This will be further explored to determine the mechanisms responsible for this synergy and identify transcriptional and epigenetic drivers and biomarkers of treatment resistance. We will launch a phase I/II clinical trial to target hypoxia concomitant with the current standard of care for men with CRPC to counteract NE trans-differentiation and prolong survival.

Project 4 (Brooks and Milosevic) will build directly on our previous studies showing that hypoxia and radiation treatment upregulate immunomodulatory cell-cell communications leading to intra-tumoral myeloid cell accumulation and treatment resistance, which can be reversed with specific chemokine inhibitors. Moving forward, we will identify the molecular 'triggers' responsible for this increase in local tumour innate immunity, the mechanism by which myeloid cell accumulation improves local treatment response and the generalizability of our finding across a range of tumour types where radiation treatment plays an important curative role. In addition, we will translate our promising laboratory findings to a biomarker-rich, multi-centre, phase I/II clinical trial in patients with cervical cancer.

Overall, our Program will lead to an increased understanding of how the spontaneous and treatment-induced tumour microenvironment *triggers* adverse cancer phenotypes that drive poor patient outcomes. Each project aims to identify novel treatments that *target* these mechanisms, several of which will be implemented clinically within this funding period. We expect our Program to go *beyond* the current paradigms of how we think about the tumour microenvironment and the unique challenges that it imposes to expose new vulnerabilities that can be exploited using novel treatments. We expect our program to have a direct impact on the clinical care of patients with brain, pancreatic, prostate or cervical cancers, and possibly other malignancies.

The Terry Fox New Frontiers Program Project Grant: Delineating therapeutic opportunities in triple-negative breast cancer

(2016-2019)

Investigators: Mathieu Lupien, Cheryl Arrowsmith, Dave Cescon, Lilian Siu, Benjamin Haibe-Kains, Linda Penn, Trevor Pugh, Pam Ohashi; Princess Margaret Cancer Centre

Co-Funder: Canadian Institutes for Health Research, Institute for Cancer Research

Scientific Summary: Breast cancer (BCa) is the second leading cause of cancer-related death in North American women. It consists of different subtypes, including Luminal A, B, HER2-positive and Triple Negative (TNBC). Biomarker-guided precision medicine strategies are applied to Luminal A/B and HER2-positive BCa patients through the use of endocrine and HER2-targeted therapies, respectively. However, this does not apply to TNBC patients. The only approved option for these patients remains cytotoxic chemotherapy. While TNBC are often regarded as chemo-sensitive, intrinsic and acquired clinical drug resistance are major problems, and coupled with early and aggressive metastatic dissemination lead to rapid progression and death in many patients. Hence, new and effective approaches to treat TNBC patients are urgently needed.

Program Goals: The overarching goal of our program is to identify targeted therapeutic approaches to improve outcome in TNBC patients by supporting basic and clinical research on immuno- (Project 1), epigenetic (Project 2) and metabolic (Project 3) therapies. Furthermore, we offer a personalized Patient-Derived Xenografts (pPDX) avatar project (Project 4) set in the context of a clinical drug development program to improve patient matching to therapies. These projects benefit from a Genomics Core developing technologies improving biomarker identification and a Patient-Derived Model Core providing physiologically relevant models for preclinical validation of new therapies. Overall, we expect our program to improve outcome of TNBC patients by identifying new therapeutic opportunities that will be directly tested in disease-relevant Patient-Derived Models. This will accelerate the translation of our discoveries by guiding future clinical studies.

Recent Discoveries and Accomplishments:

- mRNA isoforms can serve as biomarkers predictive of drug response in vitro.
- Breast cancer subtyping improvements using a pathway-based classifier.
- New and improved drug classifier to infer large-scale drug taxonomy.
- Primary TNBC demonstrate vulnerabilities to epigenetic therapy.
- Primary TNBC demonstrate sensitivities to metabolic disruptors.
- Interim reporting on the Phase II trial, biomarker-driven study of pembrolizumab (anti-PD-1) (INSPIRE; NCT02644369)

List of Key Publications:

1. Wu Q, Heidenreich D, Zhou S, Ackloo S, Krämer A, Nakka K, Lima-Fernandes E, Deblois G, Duan S, Vellanki RN, Li F, Vedadi M, Dilworth J, Lupien M, Brennan PE, Arrowsmith CH, Müller S, Fedorov O, Filippakopoulos P, Knapp S. (2019). A chemical toolbox for the study of bromodomains and epigenetic signaling. *Nat Commun*. 10:1915.
2. Clouthier DL, Lien SC, Yang SYC, Nguyen LT, Manem VSK, Gray D, Ryczko M, Razak ARA, Lewin J, Lheureux S, Colombo I, Bedard PL, Cescon D, Spreafico A, Butler MO, Hansen AR, Jang RW, Ghai S, Weinreb I, Sotov V, Gadalla R, Noamani B, Guo M, Elston S, Giesler A, Hakgor S, Jiang H, McGaha T, Brooks DG, Haibe-Kains B, Pugh TJ, Ohashi PS, Siu LL. (2019). An interim report on the investigator-initiated phase 2 study of pembrolizumab immunological response evaluation (INSPIRE). J Immunother Cancer. 7, 72.
3. Tu WB, Shiah Y-J, Lourenco C, Mullen PJ, Dingar D, Redel C, Tamachi A, Ba-Alawi W, Aman A, Al-Awar R, Cescon DW, Haibe-Kains B, Arrowsmith CH, Raught B, Boutros PC, Penn LZ. MYC Interacts with the G9a Histone Methyltransferase to Drive Transcriptional Repression and Tumorigenesis. (2018). Cancer Cell. 34, 579–595
4. Thu KL, Silvester J, Elliott M, Ba-alawi W, Elia A, Duncan MH, Mer AS, Smirnov P, Safikhani Z, Haibe-Kains B, Mak TW, Cescon DW. (2018). *Disruption of the anaphase-promoting complex confers resistance to TTK inhibitors in triple-negative breast cancer. Proc Natl Acad* Sci U S A. 115, E1570-E1577
5. Haibe-Kains B, Cescon DW. (2018). *Gene Expression Analyses in Breast Cancer: Sample Matters*. JNCI Cancer Spectr. 2, 2. https://doi.org/10.1093/jncics/pky019
6. Deblois G, Tonekaboni SAM, Kao Y, Tai F, Liu X, Ettayebi I, Grillo G, Guilhamon P, Ba-alawi W, Fedor A, Murison A, Cescon DW, Arrowsmith C, DeCarvalho D, Park M, Haibe-Kains B, Locasale JW, Lupien M. (2018). *Metabolic adaptations underlie epigenetic vulnerabilities in chemoresistant breast cancer. BioRxiv* doi: 10.1101/286054
7. Safikhani Z, Smirnov P, Thu KL, Silvester J, Lupien M, Mak TW, Cescon D, Haibe-Kains B. *Gene isoforms as expression-based biomarkers predictive of drug response in vitro*. (2017). *Nat Commun*. 8, 1126
8. Smirnov P, Kofia V, Maru A, Freeman M, Ho C, El-Hachem N, Adam GA, Ba-alawi W, Safikhani Z, Haibe-Kains B. (2017). *PharmacoDB: an integrative database for mining in vitro anticancer drug screening studies*. *Nucleic Acids Res*. 46, D994-D1002.
9. El-Hachem N, et al. (2017). Integrative cancer pharmacogenomics to infer large-scale drug taxonomy. *Cancer Res*. 77, 3057-3069.
10. Clouthier DL, Ohashi PS. (2017). Costimulation, a surprising connection for immunotherapy. *Science*. 355, 1373-1374.
11. Cescon D, Siu LL. (2017). Cancer Clinical Trials: The Rear-View Mirror and the Crystal Ball. *Cell*. 168, 575-578.
12. Crome, S.Q., et al (2017). A distinct innate lymphoid cell population regulates tumour-associated T cells. *Nat Med*. 23,368-378.
13. Barsyte-Lovejoy D, et al. (2016). Chemical Biology Approaches for Characterization of Epigenetic Regulators. *Methods Enzymol*. 574, 79-103.

The Terry Fox New Frontiers Program Project in Li-Fraumeni Syndrome: Applying genetic determinants of cancer risk to cancer surveillance and prevention

(2018-2023)

Investigators: David Malkin, Adam Shlien, Anna Goldenberg, Andrea Doria, Hospital for Sick Children, University of Toronto; Jason Berman, IWK Health Centre, Dalhousie University

Scientific Summary: Li-Fraumeni Syndrome (LFS) is a highly penetrant autosomal dominantly inherited predisposition syndrome associated with a remarkably heterogeneous presentation of early onset cancers. In 1990, the PI (Malkin) discovered that germline TP53 mutations cause >80% of LFS. Since then, this group has demonstrated that TP53 mutations occur with striking frequency in a wide spectrum of patients with component LFS tumours, with or without a family history of cancer.

In addition, epigenetic, genomic and genetic events have been found to modify the phenotypic effects of an underlying germline TP53 mutation. We have elucidated a mechanistic model to explain tumour initiation/progression in LFS in which a constitutional (or possibly early somatic) TP53 mutation favours accumulation of epigenetic or genetic events that facilitate accelerated telomere attrition, chromothripsis, and subsequent somatic cell transformation. We developed a clinical surveillance protocol that takes advantage of innovative imaging techniques such as rapid sequence whole body MRI (WB-MRI), ultrasonography and biochemical tests to detect occult malignancy. This approach improves survival and reduces treatment-related morbidity. Our protocol has been rapidly adopted worldwide.

Notwithstanding this extraordinary progress, patients with LFS continue to face seemingly insurmountable challenges:

1) It is impossible to prevent cancers from developing;

2) It is impossible to prevent therapy-induced cancers from developing;

3) It is impossible to predict what types of cancer will develop and at what age; and

4) It is extremely difficult to effectively treat these patients who face dismal survival rates with devastating treatment-related toxicities.

We propose to address these challenges through the following interwoven projects:

Projects 1-2: We will define the epigenetic and genetic modifiers that confer specific phenotypes in TP53 mutation carriers and create multi-level algorithms merging genetic and clinic-pathologic data to refine tumour type and age of onset risk estimates in TP53 mutation carriers.

Project 3: We will use this information to refine and implement novel MRI-based surveillance strategies, and to create molecular surveillance techniques for early tumour detection.

Project 4: We will explore potential chemoprevention strategies for TP53 mutation carriers using a powerful zebrafish p53 model.

Each project complements the others and addresses a key element of the continuum from molecular genotyping through risk stratification to translational strategies for early tumour detection and cancer prevention in Li-Fraumeni Syndrome. We anticipate that these studies will lead to a better understanding of the role of early p53 alterations in cancer generally, and to transform the care of patients with Li-Fraumeni Syndrome.

The Terry Fox New Frontiers Program Project Grant in targeting metabolic vulnerabilities in cancer

(2019-2025)

Investigators: Peter Siegel[1] (Project Leader), Julie St-Pierre[2] (Project Co-Leader), William J. Muller[1], Arnim Pause[1], Nahum Sonenberg[1], Vincent Giguère[1], Daniela Quail[1], Daina Avizonis[1], Lawrence Kazak[1], Ivan Topisirovic[3], Michael Pollak[3], Russell Jones[4], Frédérick Mallette[5]. [1]Goodman Cancer Research Centre and McGill University; [2]University of Ottawa; [3]Lady Davis Institute, McGill University; [4]Van Andel Institute; [5]Université de Montréal/Maisonneuve-Rosemont Hospital Research Center

Co-Funders: Goodman Cancer Research Centre, McGill University; University of Ottawa; The Quebec Breast Cancer Foundation

Scientific Summary: Metabolic reprogramming is required during tumourigenesis not only to meet the energetic and biosynthetic demands of uncontrolled growth, but also to survive under conditions of metabolic stress. *Metabolic flexibility* refers to the engagement of diverse, adaptive metabolic programs to ensure survival and enable sustained proliferation when facing environmental or therapeutic stressors. As part of our previous Program Project Grant, we uncovered critical roles for metabolic plasticity in tumour progression, metastasis and therapeutic resistance. Our renewed Terry Fox New Frontiers Program Project Grant in Targeting Metabolic Vulnerabilities in Cancer will explore this concept in much greater depth, delineating the signaling pathways and metabolic networks engaged by cancer cells in response to local changes in primary tumour and metastatic microenvironments, whole organism-level changes (obesity) and therapy-induced stresses. We will pursue four inter-related research projects that will 1) delineate metabolic adaptations that promote organ-specific metastasis, 2) define oncogene-dependent metabolic adaptations that occur during metastatic progression, 3) investigate the cross-talk between mRNA translational control and metabolic programs in cancer and 4) explore the influence of the systemic metabolic milieu on breast cancer initiation, metastasis and therapeutic resistance. Collectively, these studies will reveal molecular and cellular mechanisms conferring metabolic plasticity that drives aggressive cancer phenotypes. Ultimately, our goal is to identify metabolic "bottlenecks" or vulnerabilities that can be exploited to develop therapeutic strategies to overcome metabolic plasticity in cancer.

Recent Discoveries and Accomplishments:

- Identified distinct metabolic states of breast cancer cells underlying metastatic tropism to different sites, including a HIF-1a/PDK1-dependent glycolytic program in liver metastasis and PGC-1a-dependent programs in lung and bone metastasis.
  Dupuy, F., et al. (2015). PDK1-dependent metabolic reprogramming dictates metastatic potential in breast cancer. Cell Metabolism, 22(4):577-589. TFNF PPG PIs: St.-Pierre, J., Jones, R.G. and Siegel, P.M.
  Andrzejewski, S., et al. (2017). PGC-1a promotes breast cancer metastasis and confers bioenergetic flexibility against metabolic drugs. Cell Metabolism. 26(5): 778-787. TFNF PPG PIs: Siegel, P.M. and St-Pierre, J.
- Showed that the mTORC1/4E-BP pathway modulates synthesis of mitochondrial proteins to maintain energy homeostasis and of MTFP1 to promote mitochondrial fission.
  Morita, M., Prudent, J., et al. (2017). mTOR controls mitochondrial dynamics and cell survival via MTFP1. *Cell Metabolism,* 67(6): 922-935. TFNF PPG PIs: St-Pierre, J., Topisirovic, I., Sonenberg, N.
- Described transcriptional repression of one-carbon metabolism by PGC-1a/ERRa downstream of AMPK activation, increasing sensitivity to the anti-folate drug methotrexate.
  Audet-Walsh, E., Papadopoli, D., et al. (2016). The PGC-1a/ERRa axis represses one-carbon metabolism and promotes sensitivity to anti-folate therapy in breast cancer. *Cell Reports,* 14: 920-931. TFNF PPG PIs: Giguère, V., St-Pierre, J.
- Showed that R-2-hydroxyglutarate (2HG), produced by mutant IDH1/2, activates mTOR by inactivating KDM4A, a lysine demethylase that stabilizes its negative regulator DEPTOR.
  Carbonneau, M., et al. (2016). The oncometabolite 2-hydroxyglutarate activates the mTOR signaling pathway. *Nature Communications,* 7: 12700. TFNF PPG PIs: Jones, R.G., Topisirovic, I., Mallette F.A.
- Identified two subclasses of 5'UTRs in breast cancer cells that confer mTOR-dependent translation, differing in length and dependence on the RNA helicase eIF4A.
  Gandin, V., Masvidal, L., Hulea, L., et al. (2016). nanoCAGE reveals 5'UTR features that define specific modes of translation of functionally related mTOR sensitive mRNAs. *Genome Res.* 26(5): 636-648. TFNF PPG PIs: Pollak, M., St-Pierre, J., Topisirovic, I.
- Identified FLCN, an evolutionarily conserved negative regulator of AMPK, as a key regulator of metabolic plasticity in cancer cells, adipocytes and innate immune cells.
  Yan, M., et al. (2016). Chronic AMPK activation via loss of FLCN induces functional beige adipose tissue through PGC-1a/ERRa. *Genes & Development.* 30:1034-46. TFNF PPG PIs: St-Pierre, J., Giguère, V., Pause, A.
  El-Houjeiri, L., Possik, E., et al. (2019). The Transcription Factors TFEB and TFE3 Link the FLCN-AMPK Signaling Axis to Innate Immune Response and Pathogen Resistance. *Cell Reports* 26 (13): 3613-3628. e6. TFNF PPG PIs: Jones, R.G., Pause, A.
- Discovered a nuclear function for mTOR that correlates with poor outcome in prostate cancer and activates transcription of a gene network that enhances glycolysis and OXPHOS.
  Audet-Walsh, E., et al. (2017). Nuclear mTOR dictates androgen receptor-mediated metabolic reprogramming in prostate cancer. *Genes & Development,* 31: 1228-1242. TFNF PPG PIs: Giguère, V.
- Showed that resistance to kinase inhibitors involves mTORC1-dependent non-essential amino acid synthesis and HIF-1a-dependent glutamine metabolism.
  Hulea, L., Gravel, S.P., Morita, M., et al. (2018). Translational and HIF-1a-Dependent Metabolic Reprogramming Underpin Metabolic Plasticity and Responses to Kinase Inhibitors and Biguanides. *Cell Metab*.pii: S1550-4131(18)30566-7. TFNF PPG PIs: Avizonis, D., Siegel, P., Jones, R.G., Muller, W.J., St-Pierre, J., Pollak, M., Topisirovic, I
- Determined that metabolic flexibility in pro-metastatic neutrophils, including glutamate and proline catabolism during glucose starvation, facilitates the formation of liver metastases.
  Hsu,B., et al. (2019). Immature Low-Density Neutrophils Exhibit Metabolic Flexibility that Facilitates Breast Cancer Liver Metastasis. *Cell Reports* 27 (13):3902-3915.e6. TFNF PPG PIs: Jones, R.G., St-Pierre, J., Siegel, P.M.
- Found that the tyrosine kinase c-Src suppresses energy stress by stimulating OXPHOS, permitting mTOR-dependent translation of key epigenetic regulators of tumour progression.
  Smith, H.W., et al. (2019). An ErbB2/c-Src axis links bioenergetics with PRC2 translation to drive epigenetic reprogramming and mammary tumorigenesis. *Nature Communications*;10(1):2901. TFNF PPG PIs: Giguère, V., Topisirovic, I., Muller, W.J.

The Terry Fox New Frontiers Program Project Grant: Targeting clonal heterogeneity in treatment-refractory glioblastoma with novel and empiric immunotherapies

(2016-2019)

Investigators: Sheila Singh, McMaster University; Jason Moffat, University of Toronto and Kevin Henry, University of Ottawa.

Scientific Summary: Glioblastoma (GBM) is the most common malignant primary adult brain tumour, characterized by extensive cellular and genetic heterogeneity. Even with surgery, chemotherapy with the alkylating agent temozolomide (TMZ), and radiation, tumour re-growth and patient relapse are inevitable. Patients face a median survival of <15 months, with uniformly fatal outcomes upon disease progression post-therapy. The overall goal of our TFRI PPG is to identify new therapeutic targets that drive clonal evolution in treatment-refractory GBM, develop novel and empirical immunotherapeutic paradigms, and undertake preclinical evaluation of candidate therapeutic antibodies using our unique in vivo models of human GBM recurrence. Ultimately, we will generate a translational pipeline from initial target discovery, through target validation and mechanistic exploration, to building new biotherapeutics against novel cancer targets, and preclinical testing in our patient-derived xenograft model of treatment-resistant GBM. A promising lead panel of biotherapeutic modalities will ultimately be translated into early clinical development, generating targeted therapies and hope for future GBM patients.

Recent Discoveries and Accomplishments:

Ephrin Receptor A2/A3 (EphA2/A3): A bispecific antibody was built for dual targeting of EphA2+/A3+ GSC-enriched GBM cell populations (Qazi et al, Clinical Cancer Research, 2018). **Prominin 1 (PROM1/CD133):** A first-ever comparison multiple immunotherapeutic modalities using a novel epitope of CD133, a known GBM recurrence marker; (Vora et al, 2019, Cell Stem Cell, under revision); lead to multiple collaborations (J. Valliant, J. Bramson, R. Wylie). **Carbonic anhydrase 9 (CAIX):** Promising preclinical data showing efficacy of a CAIX BiTE in GBM: sparked interest at 2018 SNO meeting; highlighted importance of studying hypoxic TME. **Target 4:** The double jeopardy scenario of targeting both GBM cells and T cells residing in the TIME sparked our interest in this campaign; industry partner Versant sponsored an SRA providing leveraged funds; promising preclinical data on CAR T cell for rGBM presented at multiple meetings including AACR Meeting for Engineered Cell Therapies 2019 and BioCanRx. **Target 5:** The first campaign in partnership with Dr. K. Henry with expected functional redundancy similar to that of EphA2/3. **Target 6:** rGBM-specific hit emerged from CRISPR screening; collaboration with University of Virginia for selective target inhibitor, with future plans to test in combination with one of our other immunotherapies (Chokshi et al 2019, in preparation). **Target 7:** A hit targeting complement activation emerging from CRISPR screening, inhibitor obtained in collaboration with Temple University, again highlights importance of targeting the TIME.

Development of Core Platforms:

Cellular barcoding: To discern clonal evolution and the patient-specific, highly variable pattern of GBM recurrence across multiple patient-derived lines: generated a high-impact manuscript for submission (Qazi et al 2019, in preparation) in collaboration with mathematicians (Dr.Sidhartha Goyal, Univeristy of Toronto). **Whole genome CRISPR screens of human GBM:** One of few programs in the world with this expertise, a product of our multi-disciplinary training program (C. Chokshi and D. Tieu, cross-mentored by Singh and Moffat) (Chokshi & Tieu et al 2019, submitted to Cell). **Glycoproteomics:** To identify, catalog and characterize dysregulated proteins in rGBM; in collaboration with Dr. Kislinger; proven clinical utility in our personalized medicine challenge; rGBM patient made informed decision to eschew a Phase 1 TGFbR1 inhibitor trial. **RNA-SEQ:** Timepoint sampling throughout therapy informed us or evolution through therapy of GBM, and informed us for the first time about minimal residual disease (MRD) in GBM, a state we can never interrogate in human patients; resulting novel manuscript pending submission. **scSEQ:** Leveraged patient donation from "Addison's Wish" to undertake the single cell characterization of MRD in collaboration with Dr. Trevor Pugh (Qazi et al 2019, manuscript in preparation). **Tissue microarray (TMA):** Validating platform for proposed therapeutic targets built with leveraged funds from Longbow SRA; with help from neuropathologist Dr. Cynthia Hawkins the TMA incorporates 44 matched patient pGBM-rGBM pairs (one of the largest matched GBM datasets in the world).

List of Key Publications:

1. Qazi MA, Bakhshinyan D, Singh SK (2019) Deciphering brain tumor heterogeneity, one cell at a time. *Nat Med*. doi: 10.1038/s41591-019-0605-1
2. Brown KR, Mair B, Soste M, Moffat J (2019) CRISPR screens are feasible in TP53 wild-type cells. *Mol Syst Biol* 15:e8679. doi: 10.15252/msb.20188679
3. Chan K, Tong AHY, Brown KR, Mero P, Moffat J (2019) Pooled CRISPR-Based Genetic Screens in Mammalian Cells. *J Vis Exp*. doi: 10.3791/59780
4. Costanzo M, Kuzmin E, van Leeuwen J, Mair B, Moffat J, Boone C, Andrews B (2019) Global Genetic Networks and the Genotype-to-Phenotype Relationship. *Cell* 177:85–100. doi: 10.1016/j.cell.2019.01.033
5. Mair B, Aldridge PM, Atwal RS, Philpott D, Zhang M, Masud SN, Labib M, Tong AHY, Sargent EH, Angers S, Moffat J, Kelley SO (2019) High-throughput genome-wide phenotypic screening via immunomagnetic cell sorting. *Nat Biomed Eng*. doi: 10.1038/s41551-019-0454-8
6. Vora P, Seyfrid M, Venugopal C, Qazi MA, Salim S, Isserlin R, Subapanditha M, O'Farrell E, Mahendram S, Singh M, Bakhshinyan D, Chokshi C, McFarlane N, Dvorkin-Gheva A, Brown KR, Murty N, Moffat J, Bader GD, Singh SK (2019) Bmi1 regulates human glioblastoma stem cells through activation of differential gene networks in CD133+ brain tumor initiating cells. *J Neurooncol*. doi: 10.1007/s11060-019-03192-1
7. Hu, A.X., Adams, J.J., Vora, P., Qazi, M., Singh, S.K., Moffat, J., Sidhu, S.S., 2019. EPH Profiling of BTIC Populations in Glioblastoma Multiforme Using CyTOF. *Methods Mol. Biol.* 1869, 155–168. https://doi.org/10.1007/978-1-4939-8805-1_14
8. Nixon AML, Duque A, Yelle N, McLaughlin M, Davoudi S, Pedley NM, Haynes J, Brown KR, Pan J, Hart T, Gilbert PM, Singh SK, O'Brien CA, Sidhu SS, Moffat J (2019) A rapid in vitro methodology for simultaneous target discovery and antibody generation against functional cell subpopulations. *Sci Rep* 9. doi: 10.1038/s41598-018-37462-1
9. Qazi MA, Vora P, Venugopal C, Adams J, Singh M, Hu A, Gorelik M, Subapanditha MK, Savage N, Yang J, Chokshi C, London M, Gont A, Bobrowski D, Grinshtein N, Brown KR, Murty NK, Nilvebrant J, Kaplan D, Moffat J, Sidhu S, Singh SK (2018) Cotargeting Ephrin Receptor Tyrosine Kinases A2 and A3 in Cancer Stem Cells Reduces Growth of Recurrent Glioblastoma. *Cancer Res* 78:5023–5037. doi: 10.1158/0008-5472.CAN-18-0267

The Terry Fox New Frontiers Program Project Grant in overcoming treatment failure in lymphoid cancers

(2016-2021)

Investigator: Christian Steidl, BC Cancer

Co-Investigators: Marco Marra (BC Cancer, Genome Sciences Centre); Ryan Morin (SFU, BC Cancer, Genome Sciences Centre); David Scott (BC Cancer); Andrew Weng (BC Cancer); Sohrab Shah (UBC).

Scientific Summary: Collectively, lymphoid cancers are the fifth most common cancer, affecting patients of all ages. Despite generally effective treatments, a significant number of patients still die from disease progression or recurrence (treatment failure). Treatment failure results in high mortality and represents the single most important unmet clinical need for patients with lymphoid cancer. Incomplete insight into disease dynamics and tumour microenvironment biology, lack of suitable preclinical models for novel drug development and a paucity of fully implemented target-specific molecular tests have significantly hampered efforts to address this need. Our team has extensive expertise and strong preliminary data focused on treatment resistance across a wide spectrum of lymphoid neoplasms.

Goal and Projects: The main goal of this proposal is to understand and overcome treatment failure in lymphoid cancers. Specifically, we will identify and validate target-specific molecular tests characterizing genetic changes and molecular pathways that provide the preclinical rationale for novel drug development. Our proposal will further develop knowledge produced in the previous funding cycles and provide innovative approaches to overcome current roadblocks to clinical translation. In four interrelated subprojects (SPs) we will study disease dynamics in follicular lymphoma and diffuse large B cell lymphoma (DLBCL) (SP1), the genome and transcriptome of mantle cell lymphoma (SP2), the biology underlying relapse of DLBCL (SP3), and the tumour-microenvironment cellular interface (SP4). Maximizing synergy, we will utilize a common set of state-of-the-art experimental approaches (Genome Sciences Centre Analysis), draw from a unique biobank and clinical data resource (Specimen Acquisition Core) and build a strong pipeline of clinical test development and implementation (Translational Pathology Core).

Leveraging technical innovation, we will gain deep insight into clonal dynamics during disease progression and genetic mechanisms of treatment resistance in the context of microenvironment biology. Translation of the gained knowledge into targeted therapeutics and clinically useful tests will further consolidate the foundation for precision medicine applied to B cell lymphomas, and thus improve survival of patients with these diseases.

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1. Scott DW, Abrisqueta P, Wright GW, Slack GW, Mottok A, Villa D, Jares P, Rauert-Wunderlich H, Royo C, Clot G, Pinyol M, Boyle M, Chan FC, Braziel RM, Chan WC, Weisenburger DD, Cook JR, Greiner TC, Fu K, Ott G, Delabie J, Smeland EB, Holte H, Jaffe ES, Steidl C, Connors JM, Gascoyne RD, Rosenwald A, Staudt LM, Campo E, Rimsza LM. New Molecular Assay for the Proliferation Signature in Mantle Cell Lymphoma Applicable to Formalin-Fixed Parraffin-Embedded Biopsies. *J Clin Oncol*. 2017 May 20; 35(15): 1668-1677. PMID: 28291392.
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7. Twa DDW, Mottok A, Savage KJ, Steidl C. The pathobiology of primary testicular diffuse large B-cell lymphoma: Implications for novel therapies. *Blood Rev.* 2018 May; 32(3): 249-255. doi: 10.1016/j.blre.2017.12.001. Epub 2017 Dec 20. Review. PMID: 29289361
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The Terry Fox New Frontiers Program Project Grant: Targeting the Hippo signaling network in cancer

(2016-2021)

Investigators: Jeff Wrana, Steve Gallinger, Anne-Claude Gingras, Helen McNeill and Frank Sicheri Lunenfeld-Tanenbaum Research Institute, Sinai Health System; and Liliana Attisano, University of Toronto

Scientific Summary: Overcoming tissue size control is a critical hallmark of cancer that in animals is regulated by Hippo signaling. The Hippo pathway comprises a conserved kinase cassette in which Mst1/2 (the eponynomous "Hippo" gene in flies) activate Lats1/2 that in turn phosphorylate the transcription factors TAZ and YAP to drive their cytosolic sequestration, thus inhibiting TAZ/YAP target gene expression. Unlike classical signaling pathways, the Hippo pathway integrates diverse extrinsic and intrinsic cues to control growth and patterning and recent evidence shows a major role in cancer, as disruption of Hippo-TAZ/YAP coupling is subverted in most human solid tumours. However, despite an expanding description of Hippo's importance in cancer, how the pathway drives disease is not well understood. Our team has made major contributions to the molecular understanding of Hippo and its role in controlling cell signaling, cell fate and tissue size.

Goals and Projects: This TFRI-PPG seeks to capitalize on the complementary expertises of our team to build a synergistic program aimed at mapping and targeting the Hippo pathway in a number of cancers. To accomplish this, our program consists of three synergistic projects that include proteomics and functional screens to map the structure and function of Hippo regulatory networks, structural insights into how these networks modulate Hippo pathway activity, small molecule screens to identify inhibitors of YAP in breast, kidney and colorectal cancer. These projects are underpinned by the Core, which provides state-of-the-art proteomics and high throughput screening and imaging capacity. Overall, our PPG will yield new insights into the molecular and genetic mechanisms underlying cancer and provide novel therapeutic interventions in human cancer.

Recent Discoveries and Accomplishments:

- Discovered revival stem cells in the intestine
- Discovered a new mechanism of Ret-Fat4 epithelial-stromal crosstalk in the kidney
- Developed a new model of sarcomatoid renal cell carcinoma, which is associated with poor outcome in all human kidney cancers
- Developed tools for systematic in vivo functional genomics screening in mouse models of breast cancer
- Identified important upstream regulators of the core Hippo kinase cassette through functional genomics and interaction screens
- Uncovered molecular details and structural insight into how the Hippo kinase cassette is regulated
- Discovered a key role for the protein kinase, NUAK2, in enforcing the tumour-promoting activities of YAP/TAZ.
- Developed a lentiviral delivery platform for proximity-dependent biotinylation (BioID) to survey the Hippo pathway proximity interactome in different cellular context
- Built a comprehensive suite of tools for chemical proteomics to study the specificity of small molecules in a cellular milieu
- Developed extensive expertise in single cell transcriptomics
- Optimized protocols for the generation of mature human intestinal organoids
- Developed proteomics technologies in data analysis and visualization

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The Terry Fox New Frontiers Program Project Grant in porphysome nanoparticle-enabled image-guided cancer interventions

(2017-2022)

Investigator: Gang Zheng, Brian Wilson, and Jonathan Irish, Princess Margaret Cancer Centre, UHN

Scientific Abstract: This continuing program is based on the progress made on two complementary technology platforms in the first period of our TFRI grant, namely porphysome nanoparticles and photoacoustic imaging. Porphysomes are all-organic, non-toxic and intrinsically multifunctional nanoparticles that self-assemble from non-toxic, lipid-porphyrin conjugates. Based on our original discovery in 2011, we have developed three variants that will be optimized and validated here in preclinical tumour models and then in first-in-human trials to address several specific unmet needs in cancer management. Porphysome multi-functionality includes multimodal imaging contrast (photoacoustic, fluorescence, PET and MRI), enhancement of photothermal therapy (PTT) and high-efficiency photodynamic therapy (PDT).

Overall Goals: The program goal is to further develop porphysome-based imaging and image-guided cancer therapies, optimized and validated in appropriate pre-clinical animal tumour models, and then to translate the technologies into first-in-human minimally invasive trials. The initial target tumours have been selected in collaboration with clinical specialists (surgical oncology, therapeutic endoscopy, interventional radiology, tumour pathology) as having specific and significant unmet needs that could be addressed by our unique technology platform, and in which there is potential for high impact: prostate, thyroid and GI tract cancers. This goal will be achieved by multidisciplinary teams working in three projects with both in vivo preclinical and first-in-human studies, supported by a "Porphysome Foundry" Core that will synthesize the porphysomes under gmp conditions for human use. The projects are: 1) continued development and application of porphysome-contrast photoacoustic imaging, 2) porphysome-enabled PTT in treatment of focal prostate cancer and 3) porphysome-enabled PDT of thyroid cancer.

Expected outcomes: These are a) translational, in that the porphysomes will then be positioned to move into systematic clinical trials in a subset of the clinical applications informed by the pre-clinical results, potentially leading to clinical adoption and dissemination and b) transformational in further developing and validating porphysomes to address limitations in current interventional techniques.

Impact: A unique characteristic of porphysomes is that many different functions are enabled by the common porphyrin-lipid building block, creating a common pathway to translation into clinical trials and regulatory approval to address different unmet needs of cancer patients. If successful, porphysome-enabled interventions will provide new approaches to cancer management that will complement existing therapeutic modalities for multiple solid tumour types and stages, including but also beyond those selected as model tumours for the first-in-human studies in this program.

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10. Lim L, Mastragostino R, Ng K, Zheng G and Wilson BC, "Can Photoacoustic Imaging Quantify Surface Localized J-Aggregating Nanoparticles?", Journal of Biomedical Optics 2017, 22: 076008.
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The Terry Fox pan-Canadian early detection of lung cancer study

Terry Fox Research Institute Translational Cancer Research Project (2017-2021)

Investigators: Stephen Lam, Martin Tammemagi; BC Cancer, Brock University (Co-Directors), John Mayo, John Yee, John English, Anna McGuire (Vancouver General Hospital, UBC), Michael Brauer, Raymond Ng (University of British Columbia), Calum MacAulay, Trevor Dummer, Stuart Peacock (BC Cancer Research Centre, UBC), Cheryl Ho (BC Cancer, UBC, Sonya Cressman (BC Cancer Research Centre), Kwun Fong (Queensland University, Australia), Rayjean Hung (Mount Sinai Hospital, University of Toronto), Alain Tremblay (University of Calgary), Trevor Dummer (Dalhousie University, UBC), Karen Canfell (University of Sydney)

Co-Funders: VGH-UBC Hospital Foundation, BC Cancer Foundation and Australian NHMRC

Scientific Summary: The goal of the proposed extension project is to build on the expertise of the existing PanCan Early Detection of Lung Cancer study team and infrastructure, to partner with other countries such as Australia, UK and Hong Kong to conduct the International Lung Screen Trial (ILST) to address important missing information on lung cancer screening that is of global concern, namely, optimal selection criteria and management of small lung nodules. Two thousand individuals aged 55 to 80 years old and who either have an estimated six-year lung cancer risk of >1.51% based on the PLCOm2012 risk predication model, or >30 pack-years smoking history will be recruited in Canada. The incremental value of air pollution exposures and genetic susceptibility to improve the accuracy of the PLCOm2012 model will be tested and validated using data from the Canadian Partnership Tomorrow Generation Project, the Canadian Community Health Survey and, if needed, the National Taiwan University 10,000 never smoker lung cancer screening cohort. The extension project will also develop and apply advanced computer analytic imaging tools to identify and characterize small lung nodules ≤1 cm that are the most problematic in clinical management to determine their malignancy potential, with the goal of improving efficiency and accuracy of reading the large number of screening CT scans as well as decreasing unnecessary imaging studies or biopsies. The extension project will also fill the knowledge gaps to implement cost-effective lung cancer screening programs leading to a reduction in lung cancer morbidity and mortality in the general population.

Highlights:

- $PLCO_{m2012}$ lung cancer risk prediction tool is significantly more sensitive than age and pack years criteria such as the Canadian Task Force on Preventive Health Care and the US Preventive Services Task Force (USPSTF) in selecting high-risk individuals for low-dose CT screening.
- Sigificant association between outdoor air pollution exposure in female never smokers especially women of Asian ethnicity.
- The personalized risk-based PanCan nodule management protocol triaged the fewest people into diagnostic pathway and has the highest proportion of lung cancer compared to other screen-detected lung nodule protocols.
- Computer-assisted-diagnostic tool can save radiologist reading time, especially in those with no or very low-risk lung nodules which comprise of 87% of all screening LDCTs.
- Lung cancer screening using LDCT is highly cost-effective.

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Precision Oncology For Young peopLE (PROFYLE)

Terry Fox Research Institute Translational Cancer Research Project (2016-2021)

Investigators: David Malkin, Adam Shlien, Michael Taylor, Meredith Irwin, Cynthia Hawkins, Anita Villani, James Whitlock, Michael Moran, Abha Gupta (The Hospital for Sick Children/University of Toronto), Conrad Fernandez, (IWK Health Centre/Dalhousie University), Daniel Sinnett (CHU Sainte-Justine/Université de Montréal), Paul Grundy, David Eisenstat (Stollery Children's Hospital/University of Alberta), Jason Berman (Children's Hospital of Eastern Ontario/University of Ottawa), Jennifer Chan (Alberta Children's Hospital/University of Calgary), Patrick Sullivan (Team Finn Foundation), Nada Jabado (Montreal Children's Hospital/McGill University), Paul Sorenson, Steven Jones (BC Cancer), Rebecca Deyell, Rod Rassekh (BC Children's Hospital/University of British Columbia)

Co-Funders: Air Canada Foundation, Coast to Coast Against Cancer Foundation, BC Children's Hospital Foundation, Team Finn Foundation, Alberta Children's Hospital Foundation, Kids Cancer Care Foundation, Alberta Cancer Foundation, CancerCare Manitoba Foundation, SickKids Foundation, Garron Family Cancer Centre, Great Cycle Challenge, Ontario Institute for Cancer Research, CHEO Foundation, Phoebe Rose Rocks Foundation, Childcan, London Health Sciences Centre, McMaster Children's Hospital, La Fondation de l'Hopital de Montreal pour Enfants, Sarah's Fund for Cedars, Fondation Charles-Bruneau, CHU Sainte-Justine Foundation, Dalhousie Medical Research Foundation, IWK Foundation, Janeway Children's Hospital Foundation.

Scientific Summary and Aims: Cancer is the leading cause of disease-related death in children, adolescents, and young adults (CAYA). Over 3,700 CAYA are diagnosed with cancer each year in Canada, one-third of whom will have refractory or metastatic disease or will relapse. Their prognosis is grim with a survival rate of less than 15%. Sadly, this rate has not changed in the last three decades. Patients ages 15 to 29 (AYA), who make up almost 10% of all cancer patients in Canada, are particularly underserved and often present late with signs of advanced disease. Most CAYA cancer survivors suffer significant late effects of therapy (or their tumour) that require lifelong care and impose an enormous burden to them, their family and the national health care system; financial costs alone approach $1.014 million per child over their lifetime.

To address this urgent medical and socioeconomic need, Terry Fox PRecision Oncology For Young peopLE (PROFYLE) was created. PROFYLE is a pan-Canadian collaboration with the overarching vision to improve outcomes for all Canadian CAYA patients with refractory, relapsed and metastatic ('hard-to-treat') cancer. This ambitious goal is being achieved through the development and implementation of the first National Precision Oncology Pipeline providing timely access to tumour molecular profiling and the aim of identifying actionable alterations including novel targeted therapeutic options in a clinically relevant timeframe for patients. PROFYLE's success will be measured by the following seamlessly integrated aims: 1) Create a national, standardized strategy for subject enrollment and high-quality sample collection; 2) Enroll over 450 patients into PROFYLE; 3)Analyze the tumour and germline genomes and transcriptomes using standardized platforms across three sequencing centres; 4) Interrogate the cancer proteome of patients using mass spectrometry technologies and with newly developed assays to create an integrated proteogenomic pipeline for PROFYLE; 5) Identify and validate biomarkers for 'hard-to-treat' cancers in liquid non-invasive biopsies, which can be used as surrogate diagnostic, prognostic and therapeutic tools; 6) Design innovative clinical trial strategies that incorporate new drugs, off-label use and 'n-of-1' studies to enable comprehensive access to therapies for all patients identified to have actionable molecular lesions; 7) Standardize reporting and recommendations from national molecular tumour boards that will share, review and discuss consolidated proteome/genome reports informing clinical actions and enrollment of patients in novel clinical trials; 8) Engineer cell and animal model systems to functionally validate the molecular signatures, actionable markers, and screen drug candidates; 9) Explore some of the ethical challenges that arise in conducting such research.

Updates: PROFYLE has continued to grow since the last update in 2017. 320 patients have been enrolled in PROFYLE from all 16 pediatric oncology programs across Canada. In addition, over 560 patients have been profiled as part of PROFYLE and the Canadian projects it has been building on (the Personalized Oncogenomics Project (POG) in Vancouver, SickKids Cancer Sequencing Program (KiCS) in Toronto, and Personalized Targeted Therapy in Refractory or Relapsed Cancer in Childhood (TRICEPS) in Montreal).

This rapid uptake by the entire Canadian pediatric oncology community and dramatic increase in enrollment numbers has been fueled by several major PROFYLE activities. These activities include, but are not limited to, a focus on working towards pan-Canada PROFYLE Research Ethics Board (REB) approvals, the creation and establishment of the PROFYLE Consortium, the scheduling of routine tele/video conference calls to allow for communication between all the members/groups who are involved in PROFYLE across the country, the development and launch of the PROFYLE clinical data repository, and the establishment of PROFYLE regional biobanks.

Results from the first set of PROFYLE patients whose information has been analyzed show that 82% had an informative finding from their molecular profiling that had the potential to refine or alter the diagnosis, prognosis, treatment, or management of their disease. Significantly, 71% of the patients had a finding that resulted in a disease management recommendation being made by the molecular tumour boards to the patient's treating oncologist.

The PROFYLE program continues to make significant progress in other areas as well. The leadership of PROFYLE have worked to expand the PROFYLE network by engaging in communication with pharmaceutical companies and initiating discussions with international partners thereby laying the groundwork for partnerships and collaborations on a go-forward basis. These international collaborations are aiding in expanding the scope of work possible by joining forces in many areas including, but not limited to, clinical trial development, preclinical modeling including the evaluation of therapeutic responses, biomarker analyses, proteomics, and genomics and bioinformatics. Progress towards improving drug access for CAYA patients in Canada has been made through investigation of the genomic and transcriptomic results, identification of priority areas, and submission of letters of intent for clinical trial development to pharmaceutical companies. In addition, strategic collaborations with other pediatric precision medicine programs and clinical trials co-operative groups have been initiated to facilitate greater access to trials for Canadian patients, and to ensure the success of novel Canadian trial endeavours.

List of Key Publications:

- Khater K., Vairy S., Langlois L., Dumoucel S., Sontag T., St-Onge P., Bittencourt H., Dal Soglio D., Champagne J., Duval M., Leclerc J-M., Laverdiere C., Hoa Tran T., Patey N., Ellezam B., Perreault S., Piché N., Samson Y., Teira P., Jabado N., Michon B., Brossard J., Marzouki M., Cellot S., Sinnett D. (2019) Molecular profiling of hard-to-treat childhood and adolescent cancers. *JAMA Netw Open*. Apr 5;2(4): e192906.
- Khater F., Langlois S., Cassart P., Lajoie M., Healy J., Richer C., St-Onge P., Piché N., Perreault S., Cellot S., Marzouki M., Jabado N., Sinnett D. (2019) A recurrent somatic BRAF insertion (p.V504_R506dup) inducing MAPK activation and conferring resistance to RAF inhibitors in pilocytic astrocytoma. *Oncogene*. Apr;38(16):2994-3002
- Gomes C.C., Gayden T., Bajic A., Harraz O.F., Pratt J., Nikbakht H., Diniz M.G., Castro W.H., St-Onge P., Sinnett D., Mikael L.G., DeJay N., Kleinman C.L., Valera E.T., Bassenden A.V., Berghuis A.M., Majewski J., Nelson M.T., Gomez R.S., Jabado N. (2018) TRPV4, KRAS and FGFR1 gain-of-function mutations drive giant cell lesions of the jaw. *Nature Communications,* Nov 01;9(1):4572.
- Khater F., Lajoie M., Langlois S., Healy J., Cellot C., Richer C., Beaulieu P., St-Onge P., Saillour V., Minden M., Krajinovic M., Bittencourt H., Sinnett D. (2017) KMT2E-ASNS: A novel relapse-specific fusion gene in high-risk early T-cell precursor acute lymphoblastic leukemia. *BLOOD.* Mar 23; 129(12): 1729-1732

Immunotherapy network (iTNT): targeting ovarian cancer

(2016-2021)

Investigators: Pam Ohashi, UHN; Brad Nelson, BC Cancer; Marcus Butler, UHN; Benjamin Haibe-Kains, UHN; Naoto Hirano, UHN; Rob Holt, BC Cancer; Réjean Lapointe, CRCHUM; Claude Perreault, IRIC; Trevor Pugh, UHN; Lillian Siu, UHN; John Stagg, CRCHUM; Jeanette Boudreau, Dalhousie University (TFRI New Investigator).

Scientific Summary: Immunotherapy is rapidly changing the face of clinical care for cancer patients based on a plethora of promising new strategies that harness the power of the patient's own immune system to treat their cancer. Leveraging the talent, expertise and energy of Canadian scientists and clinicians, we have assembled a pan-Canadian Immunotherapy Network (iTNT) that aims to develop a deeper understanding of the immuno-biology within the tumour microenvironment and to conduct innovative clinical trials. Our initial focus is high-grade serous ovarian carcinoma (HGSOC), a challenging disease in urgent need of new treatments. We are using state-of-the-art immunological, proteomic, bioinformatic and genomic approaches to explore changes in the immune response and tumor microenvironment as patients undergo standard treatments and novel immunotherapies. We are working collaboratively to uncover the biological mechanisms that promote or inhibit anti-tumour immunity, taking into account the molecular and genetic subtypes of ovarian cancer. We are leveraging these insights to develop new combination therapies that circumvent the numerous inhibitory mechanisms that tumours exploit to evade the immune system.

Aims:

1) Comprehensive understanding of the impact of PD-1 targeted checkpoint blockade and standard chemotherapy on the immune profile and tumour microenvironment.
2) Potential identification of new signatures and biomarkers to guide subtype-directed immunotherapies.
3) Ranking of key inhibitory mechanisms in relation to the genetic and molecular subtypes of HGSC.
4) Potential identification of novel target antigens for cancer vaccines and adoptive T cell therapy.
5) Enhancing collaboration between Canadian centres developing T cell-based therapies.

Highlights:

- Completed genomic and immune profiling of 21 patients treated with pembrolizumab.
- Developed an optimized tissue and blood processing workflow for in-depth correlative studies and identified practical issues to consider when designing these studies.
- Conducted an integrated clinical, exome, and transcriptome analysis of 41 primary tumours from HGSOC patients with long-term (LT) and short-term (ST) survival and identified clinical and tumour molecular biomarkers associated with exceptional clinical response or resistance.
- Developed a subtype classifier that represents the consensus of HGSOC subtypes, an important step in defining the underlying biology and identifying therapeutic targets of HGSOC.
- Performed a bioinformatics-based, pan-cancer (incl. HGSOC) analysis of gene expression patterns associated with immunologically "cold" tumours. This revealed a strong negative relationship between the degree to which tumours have a stem cell-like phenotype and the extent of immune cell infiltration.
- Completed IHC staining and scoring of a 42-case "intratumoral heterogeneity (ITH)" HGSOC cohort, which revealed that TIL patterns can vary widely within individual patients and are influenced by the genomic and cellular architecture of tumours.
- Developed a proteogenomic strategy designed to discover mutated tumour-specific antigens (mTSAs) and aberrantly expressed TSAs (aeTSAs) coded by all genomic regions and identified noncoding regions as a major source of TSAs.
- Held international workshop on clinical application of TIL Therapy in collaboration with BioCanRx in 2016.
- Organized adoptive cell therapy (ACT) workshop to share plans, progress, and challenges in ACT research across Canada in 2017.

List of Key Publications:

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- Yang SYC, Lheureux S, Karakasis K, Burnier JV, Bruce JP, Clouthier DL, Danesh A, Quevedo R, Dowar M, Hanna Y, Li T, Lu L, Xu W, Clarke BA, Ohashi PS, Shaw PA, Pugh TJ, Oza AM. Landscape of genomic alterations in high-grade serous ovarian cancer from exceptional long- and short-term survivors. *Genome Med.* 2018 Oct 31;10(1):81.
- Zhang AW, McPherson A, Milne K, Kroeger DR, Hamilton PT, Miranda A, Funnell T, Little N, de Souza CPE, Laan S, LeDoux S, Cochrane DR, Lim JLP, Yang W, Roth A, Smith MA, Ho J, Tse K, Zeng T, Shlafman I, Mayo MR, Moore R, Failmezger H, Heindl A, Wang YK, Bashashati A, Grewal DS, Brown SD, Lai D, Wan ANC, Nielsen CB, Huebner C, Tessier-Cloutier B, Anglesio MS, Bouchard-Côté A, Yuan Y, Wasserman WW, Gilks CB, Karnezis AN, Aparicio S, McAlpine JN, Huntsman DG, Holt RA, Nelson BH, Shah SP. Interfaces of malignant and immunologic clonal dynamics in ovarian cancer. *Cell*. 2018 Jun 14;173(7):1755-1769

Multiple Myeloma Molecular Monitoring (M4) Cohort Study

Terry Fox Research Institute Translational Cancer Research Project (2017-2022)

Investigators: Tony Reiman (Project Leader; University of New Brunswick), Donna Reece (Princess Margaret Cancer Centre), Nizar Bahlis (University of Calgary), François Bénard (BC Cancer), Suzanne Trudel (Princess Margaret Cancer Centre). Co-Investigators: Aldo Del Col (Myeloma Canada), Trevor Pugh (University of Toronto), Rodger E. Tiedemann (Princess Margaret Cancer Centre), Matthew Cheung (Sunnybrook), Jonathan Sussman (Juravinski Cancer Centre), Chris Venner (Cross Cancer Institute)

Funding Partner: Myeloma Canada Research Network

Scientific Summary: Multiple myeloma (MM) is an incurable, debilitating, fatal bone marrow cancer. It is characterized by the accumulation of malignant, monoclonal plasma cells in the bone marrow with patient-specific arrangements of immunoglobulin heavy and light chain genes. Current standard treatment algorithms are increasingly effective, but are also toxic, resource-intensive, "one-size-fits-all" approaches that do not account well for individual variations in disease biology. It is crucial to understand the characteristics of MM cells that survive therapy and meditate relapse, and how to best target these cells. Standard disease monitoring techniques are dated and do not adequately detect minimal residual disease (MRD) following highly effective modern therapy. More sensitive MRD assays are promising, but evidence is lacking regarding their use in clinical decision-making. We will collaboratively apply leading-edge technologies for myeloma characterization and monitoring towards improving patient care and outcomes in a more personalized fashion.

A pan-Canadian cohort of 250 newly diagnosed MM patients treated with standard frontline therapy will be enrolled over the five-year research program. Data will be collected on the clinical course, quality of life (QOL) and resource utilization of each patient over time. We will conduct follow-up 18F-fluorodeoxyglucose (FDG) positron emission tomography (PET) scans for central review for patients who have achieved a complete response. Blood and bone marrow specimens from each patient at several key time points will be collected for disease monitoring with multiparameter flow cytometry (MFC) and next generation immunoglobulin gene sequencing (IgS). We will develop and evaluate treatment protocols in which patient care decisions are based on these novel assays. The potential impact of these technologies on relapse rates, survival, QOL and the costs of care will be examined. The clinical potential of promising, novel methods for myeloma characterization and monitoring will be explored, including assays of circulating tumour DNA (ctDNA), mechanisms of drug resistance, and progenitor cell populations.

Our work with MFC, IgS and PET will immediately inform global clinical practice and will provide a platform for modern disease monitoring for both research and patient care in Canada. Our work to develop novel assays to characterize and monitor the disease will provide a unique Canadian contribution to the field. We will translate knowledge from this study by influencing change in clinical practice guidelines, through strategic involvement in a number of national and international committees; by informing future scientific activity in the global MM research community through scientific meetings and publications; and by empowering patients with information on these technologies via existing advocacy channels.

Aims:

1. To determine the optimal integration of ultra-sensitive molecular methods of monitoring myeloma disease burden into clinical practice (i.e., IgS, MFC, and FDG-PET/CT scans); 2. To identify and implement strategies for making better MM patient management decisions based on the results of ultra-sensitive disease monitoring; 3. To explore novel methods of characterizing and monitoring the biology of the myeloma clone being developed in Canadian laboratories for their potential to further individualize therapy.

At the end of the five-year term of this research program, we will deliver:

1. A validated clinical algorithm for using MFC, IgS and PET to monitor myeloma in conjunction with currently used standard methods, with analysis of the economic impact. 2. The identification of novel treatment strategies for myeloma patient subgroups defined by baseline risk profile and response to therapy with MFC, IgS and PET. 3. The initiation of one or more clinical trials or patient management protocols in which treatment decisions are based on the results of MFC, IgS and PET. 4. Initial evaluation of the clinical potential of novel assays (ctDNA profiling, drug resistance, progenitors characterization).

Progress Update: All 10 original sites, including Saint John (HHN), Halifax (QEII), Montreal (MUHC), Montreal (HMR), Toronto (PMH), Ottawa (TOH), Winnipeg (CCM), Calgary (TBCC), Edmonton (CCI), and Vancouver (VGA) are open and recruiting. Two new sites have recently signed onto the MMTA—Hamilton (JCC) and London (LHSC), and two more sites are in the process of signing onto the MMTA, including Saskatoon (SCC) and Regina (ABCC). Currently there are 47 patients enrolled across nine sites.

Since project commencement, the Reiman biobank has been established and specimens are analyzed and stored in real time. The ctDNA panel has been designed and validated, and samples are currently being sent to begin this portion of analysis. MFC analysis occurs in real time with biospecimen collection. Clinical data is entered into the MCRN clinical outcomes database in real time. Clinical trials investigating novel drug regimens that will be paired to M4 are in development. These trials will enable researchers to apply our leading-edge research platform to patients receiving these novel therapies.

List of Key Publications:

Sarty K, Robinson B, Campbell S, Reiman T, Murugesan A. (2017). Evidence review and objectives of the Terry Fox pan-Canadian Multiple Myeloma Molecular Monitoring (M4) Study. *Oncology Exchange*. 2017 Dec; 16(4).

Enhanced Pancreatic Cancer Profiling for Individualized Care (EPPIC)

Terry Fox Research Institute Translational Cancer Research Project (2018-2022)

Principal Investigators: Daniel Renouf (BC Cancer), David Schaeffer (Vancouver General Hospital), Steven Gallinger (Ontario Institute for Cancer Research), Jennifer Knox (University Health Network), Oliver Bathe (University of Calgary), George Zogopoulos (McGill University Health Centre).

Funding partners: Ontario Institute for Cancer Research, Pancreas Cancer Canada Foundation, BC Cancer Foundation, Princess Margaret Cancer Foundation, VGH & UBC Hospital Foundation

Pancreatic cancer rates are rising in Canada and over 90% of the almost 6,000 annually diagnosed patients are not expected to survive five years from diagnosis. The majority of cases of pancreatic ductal adenocarcinoma (PDAC) are detected at the locally advanced or metastatic stage, and have the poorest outcome, driving efforts to develop new strategies to combat metastatic pancreatic cancer. In the absence of clinically validated predictive biomarkers, patients are treated with chemotherapy with limited ability to predict how individual tumours will respond to different treatments. The lack of understanding of the clinically relevant subtypes of PDAC has been a major knowledge gap hindering progress in precision medicine.

The pan-Canadian Enhanced Pancreatic Cancer Profiling for Individualized Care (EPPIC) project is a multi-centre and multi-disciplinary initiative that has begun to address this challenge by subtyping metastatic PDAC through prospective tumour molecular and clinical outcome analysis in projected 400 patients. The project encompasses clinical trials including COMPASS, led by OICR, and PanGen, led by BC Cancer, and is a collaboration between cancer centres in British Columbia, Alberta, Ontario and Quebec. Through its integrative 'omics' approach including tumour genome, transcriptome, proteome and metabolome analysis, and integration with prospectively collected clinical trial grade outcome data, EPPIC will generate unprecedented, comprehensive knowledge of the prognostic and predictive subtypes of metastatic PDAC.

Key Components and Deliverables:

1. Development of a clinical trial network focused on the collection of tumour molecular and prospective clinical outcome data, through the national expansion of COMPASS and PanGen trials.
2. Development and dissemination of a knowledge bank of metastatic PDAC with 400 fully sequenced tumour samples with clinical outcome data.
3. Identification of clinical subtypes of metastatic PDAC and predictive biomarkers, and the development of a framework for individual tumour subtyping in the clinic to guide treatment decisions.
4. Potential improvement in outcomes for individual patients within the five-year time frame of the project by guiding patients into specific clinical trials in real time.

EPPIC is generating new knowledge leading to a better understanding of PDAC progression and treatment response, and the findings may drive the development of more effective therapeutic strategies. The project has the potential to accelerate personalized therapies in PDAC, and have a transformational impact on treatment strategies and outcomes for the thousands of Canadians who will be diagnosed with metastatic pancreatic cancer within the next decade.

List of Key Publications:

Karasinska JM, Topham JT, Kalloger SE, Jang GH, Denroche RE, Culibrk L, Williamson LM, Wong HL, Lee MKC, O'Kane GM, Moore RA, Mungall AJ, Moore MJ, Warren C, Metcalfe A, Notta F, Knox JJ, Gallinger S, Laskin J, Marra MA, Jones SJM, Renouf DJ and Schaeffer DF (2019). *Altered gene expression along the glycolysis-cholesterol synthesis axis is associated with outcome in pancreatic cancer.* Clin Cancer Res. 2019 Sep 3. doi: 10.1158/1078-0432.CCR-19-1543.

Jones MR, Williamson LM, Topham JT, Lee MKC, Goytain A, Ho J, Denroche RE, Jang G, Pleasance E, Shen Y, Karasinska JM, McGhie JP, Gill S, Lim HJ, Moore MJ, Wong HL, Ng T, Yip S, Zhang W, Sadeghi S, Reisle C, Mungall AJ, Mungall KL, Moore RA, Ma Y, Knox JJ, Gallinger S, Laskin J, Marra MA, Schaeffer DF, Jones SJM and Renouf DJ (2019). *NRG1 gene fusions are recurrent, clinically actionable gene rearrangements in KRAS wild-type pancreatic ductal adenocarcinoma. Clin Cancer* Res. 2019 Aug 1;25(15):4674-4681. doi: 10.1158/1078-0432.CCR-19-0191.

The Canadian Prostate Cancer Biomarker Network (CPCBN)

Terry Fox Research Institute Translational Cancer Research Project (2010-2019)

Investigators: Fred Saad, Anne-Marie Mes-Masson, Mathieu Latour, Pierre Karakiewicz, Jean-Baptiste Lattouf, Louis-Mathieu Stevens, Mathieu Latour, Dominique Trudel, John Stagg, CHUM; Marie-Paule Jammal, Cité de la Santé de Laval; Jean-Benoît Paradis, Complexe Hospitalier de la Sagamie; Armen Aprikian, Simone Chevalier, Simon Tanguay, Jacques Lapointe, Fadi Brimo, McGill University Health Centre; Louis Lacombe, Alain Bergeron, Yves Fradet, CHUQ; Neil Fleshner, Rob Bristow, Alejandro Berlin, Theodorus van der Kwast, Antonio Finelli, Shabbir Alibhai, UHN; Laurence Klotz, Margaret Fitch, Sunnybrook Hospital; Darell Drachenberg, Manitoba Prostate Centre; Martin Gleave, Ladan Fazli, Alan So, VPC; Simon Sutcliffe, BC Cancer.

Scientific Summary: Prostate cancer is the most commonly diagnosed cancer with an estimated 22,900 new cases in 2019 and it is the third leading cause of cancer-related death in Canadian men. The introduction in the 1990s of prostate specific antigen (PSA) as a screening tool greatly facilitated the diagnosis of prostate cancer and, in particular,r favoured the detection of early stage, and in some cases low-grade (Gleason 6) tumours. In patients with low-grade tumours, it is presently difficult to differentiate between low- and high-risk disease, which contributes to the overtreatment of men for whom interventional therapy is neither required, nor appropriate, to ensure a lifespan uncompromised by cancer or its therapeutic consequences. Therefore, there is an urgent need for new prognostic tools that will allow the distinction between low-grade tumours requiring definitive therapy and those that are best suited for observation. When patients are under active surveillance (AS), practitioners routinely measure PSA levels and monitor signs of disease progression through regular biopsies and digital rectal exams. This delays curative treatment in low-risk patients until there are indications that the disease is progressing, at which time active treatment is initiated. Moreover, there is also a need to identify biomarkers that will add to the currently used clinical and pathological parameters to identify patients at high-risk of cancer recurrence and/or progression that may benefit from adjuvant or neo-adjuvant therapies. This would have the potential of directing high-risk patients to multi-modal therapy and/or trials with novel therapies in order to limit their disease. Accurate and individualized risk stratification may have profound individual (lower recurrence rates, better quality of life) and societal (lower cost, better use of health resources) implications.

To accomplish its goals, the CPCBN assembled a large tissue microarray (TMA) series of 1,512 radical prostatectomy specimens associated with extensive clinico-pathological data. This important resource is presently being shared among Canadian researchers to validate biomarkers related to prostate cancer patient prognosis. In addition, the CPCBN also created a TMA series of 125 biopsy specimens from intermediate-risk patients treated by radiotherapy in combination or not with hormonotherapy. Due to the nature of biopsy specimens, this TMA will be used only for binary markers in immunohistochemistry. In collaboration with GenomeDx, the CPCBN moved towards an RNA/DNA approach for its cohort of patients treated by radiotherapy. Microarray expression data GenomeDx signature category of risk and copy number alterations from over 200 patient specimens are available for researchers. The CPCBN is presently performing an RNASeq approach for the AS cohort of 250 patients. To access the CPCBN TMA series and extracted material or profiling data, researchers must fill an application form for their proposal to be evaluated by the study committee.

The CPCBN is focused on the identification of biomarkers that predict risk in order to inform clinical management decisions. While there are clear advantages from a health/quality of life/health economic viewpoint to AS, its uptake within the Canadian context was not studied. Indeed, the extent to which it is practiced, the barriers to its implementation, and health professional/societal views on its acceptance in the Canadian context was poorly documented. Using database interrogation and chart review approaches in four different provinces (Quebec, Ontario, Manitoba, British Columbia) the CPCBN monitored AS in men that underwent a biopsy in 2010 to provide evidence for the extent of active surveillance uptake in Canada. We noted considerable regional differences in the AS uptake in Quebec. Thus, we were interested in investigating if these differences still remain six years after the initial baseline and evaluate if the uptake reflects the integration of growing knowledge and acceptance of AS as an initial management strategy. This project is ongoing. In parallel, using a focus-group approach, patients and health care providers of the same four provinces were interrogated to identify perceived barriers and facilitators to AS. This research was published recently. Ultimately, the CPCBN aims to reduce the impact of prostate cancer by incorporating key molecular information about expression, prognosis, response and outcome into algorithms defining optimized, individualized therapy. The program is also defining how best to transfer this new knowledge within the Canadian health care setting. In particular, this approach has the potential to stratify patients with low-risk disease, as determined by current criteria, into a larger group for whom no further therapy is required to achieve survival unimpeded by prostate cancer (AS) from a small group whose disease, despite being apparently low-risk, will progress and result in premature death if left untreated.

List of Key Publications:

1. Leyh-Bannurah SR, Trudel D, Latour M, et al. A Multi-Institutional Validation of Gleason Score Derived from Tissue Microarray Cores. *Pathol Oncol* Res. 2019; 25: 979-86.
2. Grosset AA, Ouellet V, Caron C, et al. Validation of the prognostic value of NF-kappaB p65 in prostate cancer: A retrospective study using a large multi-institutional cohort of the Canadian Prostate Cancer Biomarker Network. *PLoS Med.* 2019; 16: e1002847.
3. Dankner M, Ouellet V, Communal L, et al. CCN3/Nephroblastoma Overexpressed Is a Functional Mediator of Prostate Cancer Bone Metastasis That Is Associated with Poor Patient Prognosis. *Am J Pathol.* 2019; 189: 1451-61.
4. Pang K, Fitch M, Ouellet V, et al. Describing perspectives of health care professionals on active surveillance for the management of prostate cancer. *BMC Health Serv Res.* 2018; 18: 430.
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glossary

BCCA	BC Cancer Agency
BCCRC	BC Cancer Research Centre
CHUM	Centre hospitalier de l'Université de Montréal
CHUQ	Centre hospitalier de l'Université de Quebéc
CRCHUM	Centre de recherche du Centre hospitalier de l'Université de Montréal
MOHCCN	Marathon of Hope Cancer Centres Network
MSGSC	Michael Smith Genome Sciences Centre
OHRI	Ottawa Hospital Research Institute
OICR	Ontario Institute of Cancer Research
PM	Princess Margaret Cancer Centre
SFU	Simon Fraser University
TBCC	Tom Baker Cancer Centre, Calgary
UBC	University of British Columbia
UdeM	Université de Montréal
UHN	University Health Network
UofM	University of Manitoba
UofO	University of Ottawa
UofT	University of Toronto
VGH	Vancouver General Hospital
VPC	Vancouver Prostate Centre

The Terry Fox Research Institute from coast to coast

TFRI is an Institute without walls linking the capabilities of 95 leading cancer care and cancer research institutes and universities organized through six regional "nodes"

NATIONAL PARTNERS
BioCanRx
Canadian Partnership Against Cancer
Canadian Institutes of Health Research
Canadian Tumour Repository Network (CTRnet)
Genome Canada
Myeloma Canada
Pancreas Cancer Canada Foundation
The C17 Council
The Terry Fox Foundation

BRITISH COLUMBIA
adMare Bioinnovations
BC Cancer
BC Cancer Foundation
BC Children's Hospital Research Institute
Genome BC
Michael Smith Genome Sciences Centre
Simon Fraser University
St. Paul's Hospital (Providence Health)
Team Finn Foundation
TRIUMF
University of British Columbia
University of Victoria
Vancouver Coastal Health Research Institute
Vancouver General Hospital
Vancouver Prostate Centre
VGH & UBC Cancer Foundation

ALBERTA
Alberta Cancer Foundation
Alberta Children's Hospital
Alberta Health Services
Alberta Innovates – Health Solutions
Cross Cancer Centre
Genome Alberta
Tom Baker Cancer Centre
Stollery Children's Hospital
University of Alberta
University of Calgary

PRAIRIES
CancerCare Manitoba
University of Manitoba
Manitoba Prostate Centre
Saskatchewan Cancer Agency
University of Saskatchewan

ONTARIO
Brock University
Children's Hospital of Eastern Ontario Research Institute
Encycle Therapeutics
The Hospital for Sick Children
Juravinski Cancer Centre
London Health Sciences Centre
Lunenfeld-Tanenbaum Research Institute
McMaster University
Mount Sinai Hospital
Ontario Institute for Cancer Research
Ottawa Hospital Research Institute
Princess Margaret Cancer Foundation
Queen's University
Ryerson University
Sunnybrook Research Institute
St. Michael's Hospital
Trillium Health Partners
University Health Network – Princess Margaret Cancer Centre
University of Guelph
University of Ottawa
University of Toronto
Western University

QUEBEC
Centre de Recherche Hôpital Maisonneuve-Rosemont
Centre hospitalier de L'Université de Montréal
Centre hospitalier Universitaire de Québec
CHU Sainte-Justine Fondation
CHU Sainte-Justine
CSSS Champlain -- Charles Le Moyne
Jewish General Hospital
Institut de recherche en Immunologie et cancérologie
Hopital du Sacré Coeur de Montréal
Hopital Maisonneuve- Rosemont
McGill University Goodman Cancer Centre
McGill University Health Centre
McGill University
Montreal Children's Hospital
Polyclinique Medicale Concorde
Polytechnique Montréal
Quebec Breast Cancer Foundation
Royal Victoria Hospital
St. Mary's Hospital Centre
The Cole Foundation
Université de Montréal
Université Laval
Université Sherbrooke

ATLANTIC
Atlantic Cancer Research Institute (Moncton)
Beatrice Hunter Cancer Research Institute
Horizon Health Network
Dalhousie University
Dr. Georges L. Dumont University Health Centre
Eastern Health
Izaak Walton Killam Health Centre
Memorial University of Newfoundland (St. John's)
Saint John Regional Hospital
University of New Brunswick
The University of Prince Edward Island

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