The Terry Fox Research Institute
L'Institut de recherche Terry Fox

2013 version

terry fox cancer research portfolio 2013

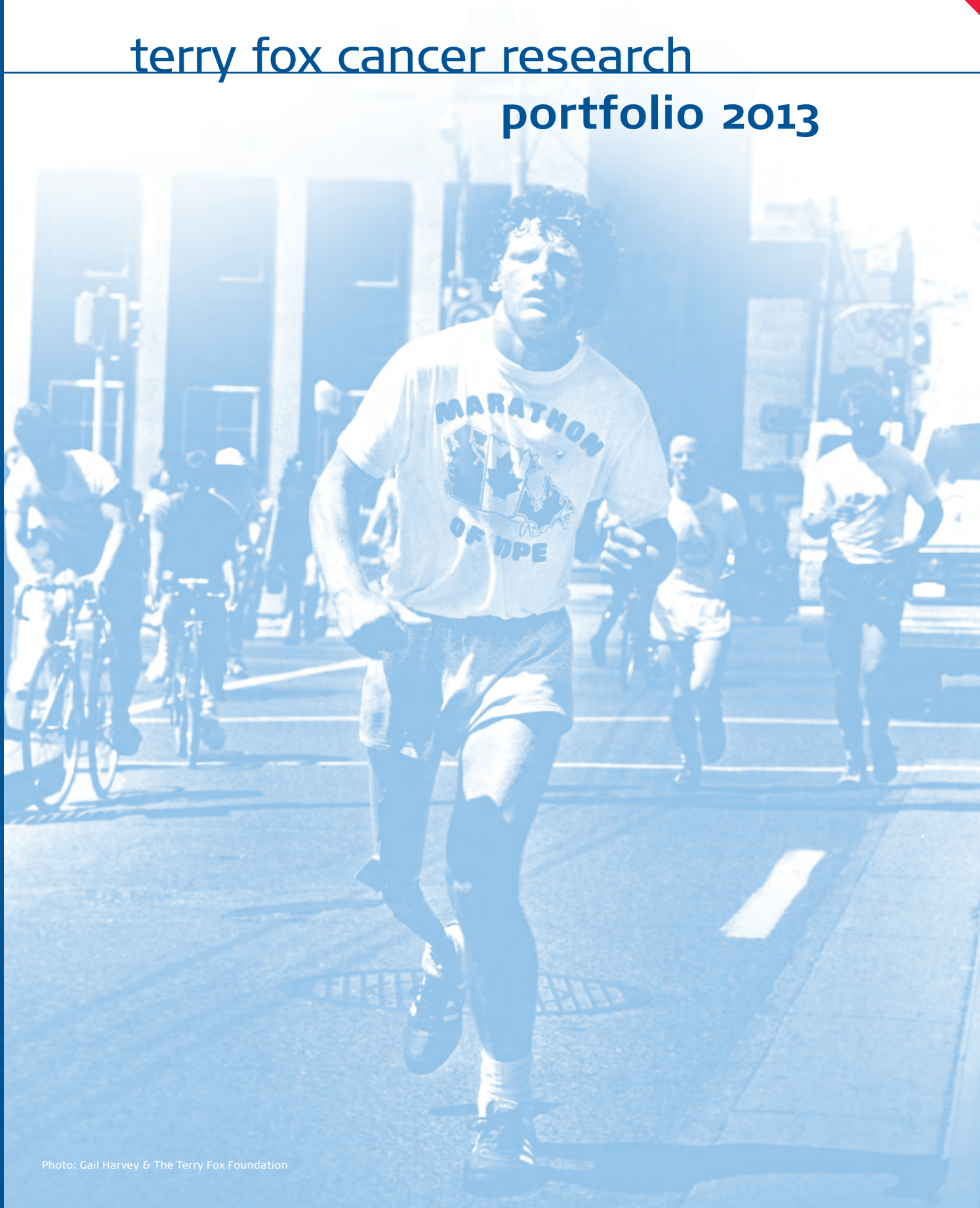

Photo: Gail Harvey & The Terry Fox Foundation

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 $600 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 Chilliwack, 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 (TFRI) www.tfri.ca

Launched in October 2007, The Terry Fox Research Institute (TFRI) is the brainchild of The Terry Fox Foundation and today acts as its research arm, overseeing its complete investment portfolio. TFRI seeks to improve significantly the outcomes of cancer research for the patient through a highly collaborative, team-oriented, milestone-based approach to research that will enable discoveries to translate quickly into practical solutions for cancer patients worldwide. TFRI collaborates with over 50 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.

Photo: James Park

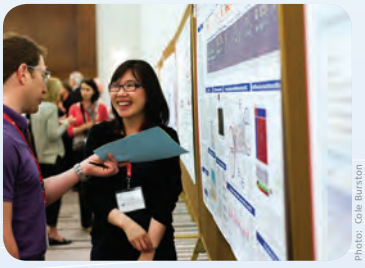

Photo: Cole Burston

Photo: James Park

Terry Fox 4th annual scientific meeting participants in Ottawa, May 2013

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 its 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 Investigator Awards These awards provide a three-year operating grant to independent cancer researchers within the first five years of their first faculty appointment. New Investigators are sponsored and mentored by an existing translational project or New Frontiers Program Project team.

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 Translational Cancer Research By invitation only, TFRI asks expert multidisciplinary teams to develop collaborative pan-Canadian research projects to align with its translational research mandate. These projects mostly target a specific cancer with a focus on moving discoveries and knowledge into practical solutions for patients within a relatively short timeframe of five to ten years. This is accomplished via an iterative process of developing milestone-driven "business plans" to focus on the outcomes impact of the research.

Terry Fox Research Training Awards TFRI partners with other Canadian funding agencies to offer support for trainees. In partnership with the Canadian Institutes of Health Research, The Terry Fox Foundation supports four integrated training programs under its Strategic Training Initiatives in Health Research (STIHR) program. TFRI also partners with specific provincial health research agencies to build research capacity.

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/


Photo: Stephane Lord/Multimedia Production, CHUM


Photo: Chuck Russell / BC Cancer Agency

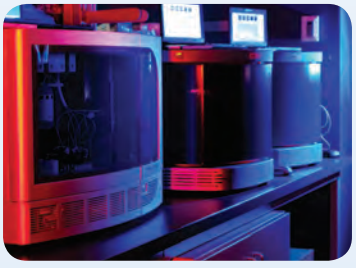
Photo: Martin Krzywinski

table of contents

Terry Fox New Investigator Operating Grants

Terry Fox New Frontiers Program Project Grants

Terry Fox Research Institute Translational Cancer Research Projects

A personalized oncolytic vaccine: Using oncolytic viruses to exploit neo-antigens derived from the tumour mutanome

Terry Fox New Investigator Operating Grant (2012-2015)

Investigator: Rebecca Auer, Ottawa Hospital Research Institute (OHRI) **Collaborator:** John Bell, OHRI

Scientific Summary: The immune system plays a central role in the cancer outcomes of most solid tumours, in particular in the eradication of micrometastatic disease following surgical resection of the primary tumour. Cancer immunotherapies must effectively target self-derived tumours while avoiding autoimmune side effects, a potentially fatal penalty for effective immunotherapy. A recent focus in the field of cancer immunotherapy is "immunomics," combining immunology with genomics to identify neo-epitopes, based on tumour-specific somatic mutations (the mutanome), with the goal of designing personalized cancer vaccines that are less likely to produce autoimmune pathology. The rapid advances in next generation sequencing means that personalized cancer vaccines will be realized in the next five years. A promising cancer vaccine platform is based on oncolytic viruses (OV). OVs selectively replicate in tumour cells resulting in immunogenic cell death. Oncolytic vaccines (OV expressing tumour antigens) provide an even more powerful boost to pre-existing antitumour immunity by combining viral oncolysis with the presentation of tumour antigens. The Terry Fox Canadian Oncolytic Virus Consortium (COVCo) is a leader in the development of oncolytic vaccines and combining this strategy with a personalized approach to antigen selection, based on the tumour mutanome represents a highly novel application of both technologies.

Hypothesis: We hypothesize that the antitumoural immunity induced by viral oncolysis can be utilized to identify immunogenic neo-antigens derived from the tumour mutanome. We further hypothesize that immunizations with oncolytic vaccines expressing these mutated sequences will result in a therapeutic antitumour immune response without development of autoimmunity and that this strategy can be successfully combined with surgical resection of the primary tumour to eradicate micrometastatic disease.

Specific Aims: To determine the effect of tumour oncolysis on the *in-vivo* generation of T cell-mediated immune responses to mutant peptide neo-antigens derived from the B16 mutanome; to assess the therapeutic and autoimmune effects of a prime-boost vaccination strategy using a recombinant adenovirus vaccine vector and a complementary replicating rhabdoviral oncolytic vaccine expressing selected mutant peptide neo-antigens derived from the B16 mutanome; and to evaluate a peri-operative, prime-boost immunization strategy to treat metastatic disease in combination with surgery in a B16 tumour model.

Methods: In this project we will make use of the murine B16 melanoma model, one of the most widely used models for scientific validation of T cell-based immunotherapies, and for which the mutanome has been published. This project will establish the pre-clinical proof of concept for a personalized oncolytic vaccination strategy that can ultimately be employed in patients following deep sequencing of their own tumour.

List of Key Publications:

1. Tai LH, Zhang J, Tanese de Sousa C, Alkayyal A, Ananth AA, Sahi S, Mahmoud AB, Bell JC, Makrigiannis A, Auer RC. Perioperative influenza vaccination reduces postoperative metastatic disease by reversing surgery-induced dysfunction in natural killer cells. *Clin Cancer Res* (epub doi 10.1158/1078-0432.CCR-13-0246). IF:7.837

2. Tai LH, Tanese de Sousa C, Rintoul J, Ly L, Zhang J, Falls TJ, Belanger S, Bell JC, Makrigiannis A, Auer RC. Preventing postoperative metastases by enhancing natural killer cell function with novel oncolytic virus therapy. *Cancer Res* 2012;73(1):97-107. IF:8.650

3. Lemay CG, Rintoul JL, Kus A, Paterson JM, Garcia V, Falls TJ, Ferreira L, Brindle BW, Conrad DP, Tang VA, Diallo JS, Arulanandam R, LeBoef F, Stojdl DF, Lichty BD, Atkins HL, Parato KA, Bell JC, Auer RC. Harnessing oncolytic virus-mediated anti-tumour immunity in an infected cell vaccine. *Mol Ther* 2012;20(9):1791-9. IF:7.041

4. Seth R, Tai LH, Falls T, Tanese de Sousa C, Bell J, Carrier M, Atkins H, Boushey R, Auer RC. Surgical stress promotes the development of cancer metastases by a coagulation-dependent mechanism involving Natural-Killer cells in a murine model. *Ann Surg* 2013; 258(1):158-68. IF:6.329

5. Rintoul JL, Lemay CG, Tai LH, Stanford MM, Falls TJ, de Souza C, Bridle BW, Ohashi PS, Wan Y, Lichty BD, Mercer AA, Auer RC, Atkins HL, Bell JC*. ORFV:A Novel Oncolytic and Immnune Stimulating Parapoxvirus Therapeutic. Mol Ther 2012;20(5):1148-57. IF:7.041

6. Auer RC, Bell JC. Oncolytic viruses: smart therapeutics for smart cancers. *Future Oncol.* 2012 Jan;8(1):1-4. IF:2.455

Flow informatics approaches for the identification of normal and malignant stem cells

Terry Fox New Investigator Operating Grant (2010-2013)

Investigator: Ryan Brinkman, BC Cancer Agency **Collaborator:** Keith Humphries, BC Cancer Agency

Scientific Summary: We are developing and applying bioinformatics approaches to assist in the analysis of high-throughput, high-dimensional flow cytometry data. A recent focus is methods development related to clustering methods for flow cytometry data to overcome the limitations of manual gating. We are applying these methods to the analysis of telomeres, hematopoietic and leukemic stem cells, ovarian cancer, leukemia and lymphoma, HIV, Parkinson's disease and tuberculosis. The application of these methodologies will improve the robustness of the current, manual-based analysis of flow cytometry data, with the aim of developing an automated analysis platform that extends from quality checking, normalization, cell population identification and feature extraction to finally sample classification (diagnosis) and biomarker identification.

Aims: To develop an automated analysis platform to support accuracy of lymphoma/leukemia sub-type diagnosis; and to provide a user-friendly lymphoma/leukemia automated analysis platform. We have addressed these through four separate sub-projects: Diffuse Large B-cell Lymphoma (DLBCL) sub-type classification; Differential classification of Mantel Cell Lymphoma (MCL) and Small Lymphocytic Lymphoma (SLL), two highly similar cancers that are often difficult to distinguish; Differential classification of DLBCL and Follicular Lymphoma (FOLL); and Distinguishing Germinal Center B-cell Lymphoma (GC-L) from Lymphoid Hyperplasia (GC-H).

Updates: We were able to build a highly accurate diagnostic aid to distinguish between MCL and SLL. The diagnostic aid is now being used by clinicians in their evaluation of difficult-to-diagnose cases at the BC Cancer Agency. We also identified a new biological marker that can aid in prognosis for DLBCL. This is of particular interest because this biomarker is readily available in most clinical laboratories without significant alteration to existing routine diagnostic strategies or incurring additional costs. We are developing a statistics-based analysis platform to improve the accuracy of sub-type diagnosis, including identification of patients that are difficult to diagnose, potentially misdiagnosed, or have an ambiguous diagnosis, and provide a user-friendly lymphoma and leukemia automated analysis platform, including a web-browser based, clinician-accessible user interface. Development of new computational tools for lymphoma and leukemia classification will improve and help identify potential data quality problems that may result in inconsistent cancer-test results while alleviating the manually intensive nature of diagnosis review.

List of Key Publications:

1. Craig F, Brinkman R, Ten Eyck S, Aghaeepour N. Computational Analysis Optimizes the Flow Cytometric Evaluation for Lymphoma. *Cytometry B* (in Press)

2. Spidlen J, Breuer K, Rosenberg C, Kotecha N, Brinkman RR. FlowRepository: A resource of annotated flow cytometry datasets associated with peer-reviewed publications. *Cytometry A* 81: 727-731, 2012. PMID: 22887892

3. Aghaeepour N, Jalali A, O'Neill K, Chattopadhyay PK, Roederer M, Hoos HH, Brinkman RR. RchyOptimyx: cellular hierarchy optimization for flow cytometry. *Cytometry A* 81: 1022-1030, 2012. PMID: 23044634

4. Streitz M, Fuhrmann S, Thomas D, Cheek E, Nomura L, Maecker H, Martus P, Aghaeepour N, Brinkman RR, Volk H-D, Kern F. The phenotypic distribution and functional profile of Tuberculin-specific CD4 T-cells characterizes different stages of TB infections. *Cytometry B* Clin Cytom 82: 360-368, 2012. PMID: 22961735.

5. Aghaeepour N, Finak G, The FlowCap Consortium, The Dream Consortium, Hoos H, Mosmann TR, Gottardo R, Brinkman RR*, Scheuermann RH*. Critical assessment of automated flow cytometry analysis techniques. *Nature Methods* 10: 228-38, 2013. PMID: 2336282

6. Zare H, Haffari G, Gupta A, Brinkman RR. Scoring relevancy of features based on combinatorial analysis of Lasso with application to lymphoma diagnosis. *BMC Genomics* 14 Suppl 1: S14, 2013. PMID: 23369194

7. Villanova F, Di Meglio P, Inokuma M, Aghaeepour N, Perucha E, Mollon J, Nomura L, Hernandez-Fuentes M, Cope A, Prevost AT, Heck S, Maino V, Lord G, Brinkman RR, Nestle FO. Integration of lyoplate based flow cytometry and computational analysis for standardized immunological biomarker discovery. PLoS One, 8;e65485. 2013. PMID: 23843942

8. Spidlen J, Barsky A, Angermann B, Wilkinson P, Breuer K ,Png A, Cortes A, Carr P, Liefeld T, Reich M, Nazaire, M-D, Eaves CJ, Mesirov JP , Sekaly RP, Brinkman RR., "GenePattern Flow Cytometry Suite", *Source Code for Biology and Medicine.* PMID 23822732

9. Aghaeepour N., Brinkman R. Computational Analysis of High-dimensional Flow Cytometric Data for Diagnosis and Discovery in Fienberg H. and Nolan G (eds.). *Current Topics in Microbiology and Immunology* (CTMI) volume on "Novel techniques of multiparametric cytometry and large scale data analysis" (In Press); PMID 23975083

10. Hills M, O'Neill K, Falconer E, Brinkman R & Lansdorp PM., "Organizing genomes and mapping rearrangements in single cells.", *Genomic Med PubMed ID*: n/a-Accepted

A novel platform for glioma modeling to accelerate the therapeutic targeting of glioblastoma

Terry Fox New Investigator Operating Grant (2011-2014)

Investigator: Jennifer Chan, University of Calgary **Funding Partner:** Alberta Cancer Foundation

Scientific Summary: The overall aim of this project is to improve the therapeutic targeting of glioblastoma (GBM) by developing a novel modeling system for the disease. Our system is based on electroporation, a technique that allows neural progenitors of embryonic or neo-natal mice to be directly targeted with genetic material such as plasmids. We have constructed a library of transposon-based plasmids carrying various oncogenic drivers such as activated Ras and EGFRViii, which we are using together with cre-expressing plasmids to generate brain tumours in mice carrying floxed tumour suppressor alleles. The resulting tumours, generated in either embryonic or neo-natal mice, show features including vascular proliferation, necrosis, diffuse growth, giant cells and mitosis, i.e., histological characteristics of human GBM. These tumours arise with high penetrance and, with most mice developing large tumours by five weeks post neo-natal electroporation, rapidly. We are currently analysing the tumour RNA profiles to determine if they resemble the well-characterized subtypes of human gliomas. We plan to use our system to examine the relative contribution of the cell type targeted and the oncogenic driver gene used to sub-type classification and to functionally validate the role of genes newly implicated with human brain tumours through sequencing. Together with other TFRI-sponsored investigators, we recently identified mutations in the transcriptional repressor CIC in a majority of oligodendrogliomas, another type of malignant glioma, and have generated a floxed CIC mouse strain to assess its contribution to normal brain development and tumourigenesis. Electroporation allows us to quickly and easily target loss of CIC to neural progenitors or their descendents alone and in combination with other genes commonly mutated in oligodendrogliomas e.g. IDH1. Thus, we have shown the effectiveness of our tumour modeling system and are well placed to demonstrate its rapid adaptability to investigate a number of key issues in brain tumour biology. The overall goals of this project are to:

- Determine if tumours generated by introduction of mutated forms of PDGFR, EGFR, and RAS into wild type and tumour suppressor deleted neural progenitors recapitulate the features of known sub-classes of gliomas. We will assess the similarity of tumours to human tumours at the protein and RNA levels. We will also assess whether the developmental stage of the targeted cell affects the elaboration of different glioma sub-types.
- Test whether the model can be used to dissect relationships between the RAS/MAPK, AKT, and mTOR signaling and STAT3 activation – and determine if specific pathway aberrations are predictive of treatment response to specific targeted agents.
- Test the biologic role of newly discovered mutations identified through the sequencing efforts of the TFRI GBM program project to discern whether they may constitute new targets for therapy.

List of Key Publications:

1. Spence T*, Perotti C*, et al. A novel C19MC-amplified cell line links Lin28/let-7 to mTOR signaling in Embryonal Tumour with Multilayered Rosettes. (In press, *Neuro-Oncology*, 2013) (*equal contribution)
2. Ramkissoon LA, et al. Genomic analysis of diffuse pediatric low-grade gliomas identifies recurrent, oncogenic MYBL1-truncating rearrangements. *Proc Natl Acad Sci* May 14;110(20):8188-93 (2013).
3. Blough MD, et al. DNA Hypermethylation and 1p Loss Silence *NHE-1* in Oligodendroglioma. *Ann Neurol* Jun;71(6):845-9 (2012).
4. Northcott PA, et al. Subgroup specific structural variation across 1,000 medulloblastoma genomes. *Nature*. Aug 2;488(7409):49-56. (2012)
5. Picard D, et al. Markers of survival and metastatic potential in childhood CNS primitive neuro-ectodermal brain tumours: an integrative genomic analysis. *Lancet Oncol*; 13(8):838-48. (2012)
6. Yip S, et al. Concurrent CIC mutations, IDH mutations and 1p/19q loss distinguish oligodendrogliomas from other cancers. *J Pathol.* Jan; 226(1):7-16. (2012)
7. Goto J, et al. Regulable neural progenitor-specific TSC1 loss yields giant cells with multi-organelle dysfunction in a mouse brain model of tuberous sclerosis complex. *Proc Natl Acad Sci USA*: Nov 8, 108(45):E1070-9 (2011).
8. Qin W*, Chan J*, et al. Analysis of TSC cortical tubers by deep sequencing of TSC1, TSC2, and KRAS demonstrates that small second hit mutations in these genes are rare events. *Brain Pathology:* Nov, 20(6):1096-105 (2010). (*equal contribution.)
9. Chan JA*, Balasubramanian S*, Witt RM*, et al. Proteoglycan interactions with Sonic Hedgehog specify mitogenic responses. *Nat Neurosci*: Apr, 12(4):409-17 (2009). (*equal contribution.)
10. de la Iglesia N, et al. Identification of a PTEN-regulated STAT3 brain Tumour suppressor pathway. *Genes Dev:* Feb 15, 22: 449-62 (2008).

Role of spatial chromatin organization in genome regulation

Terry Fox New Investigator Operating Grant (2008-2013)

Investigator: Josée Dostie, McGill University

Scientific Summary: The goal of this research project is to examine cancer from an entirely new epigenetic perspective. The term "epigenetics" refers to heritable changes in gene expression which are not associated with modifications in the primary DNA sequence. Epigenetic gene silencing is a hallmark of cancer cells and targeting of the epigenome has already yielded promising results in clinical trials for hematopoietic malignancies. Although epigenetic control is known to involve DNA methylation and post-translational histone modifications, the nature of epigenetic marks inducing aberrant gene expression remains poorly characterized. Importantly, we also know very little about the molecular mechanisms responsible for altered epigenomes in cancer cells. For that reason, a better understanding of epigenetic mechanisms is essential to develop effective strategies for diagnosis, prevention, and the treatment of cancer. The goal of my research program is to understand how epigenetic regulation can lead to changes in gene expression by altering spatial chromatin organization. In this project, we are mapping the spatial organization of the Hox gene clusters during normal hematopoiesis and in leukemia. We selected the Hox genes as models because of their pivotal roles in human biology and in various malignant diseases, including leukemia. The Hox clusters are ideal models to study how genetic and epigenetic alterations can cause improper gene expression by inducing changes in the three dimensional architecture of genomes because Hox regulation is known to involve various epigenetic mechanisms. To map the architecture of the Hox clusters, we are using the "Chromosome Conformation Capture" (3C) and 3C-Carbon Copy (5C) technologies (8-10). The 3C/5C technologies are extremely powerful approaches that enable high resolution analysis of chromatin architecture *in vivo*. We expect to identify Chromatin Conformation Signatures (CCSs) of normal and aberrant Hox expression and new mechanisms of Hox gene regulation. These signatures will represent an entirely novel class of human disease biomarker.

Specific Aims:

Spatial organization of the Hox clusters.

- Experimental design.
- Analysis of Hox clusters by the Chromosome Conformation Capture (3C) methodology.
- Analysis of Hox clusters by the Chromosome Conformation Capture Carbon Copy (5C) approach.

Spatial organization of Hox clusters in normal hematopoiesis and leukemia.

- Identification of Hox CCSs during normal hematopoiesis.
- Identification of Hox CCSs in leukemia cell models.
- Functional characterization of hematopoietic Hox CCSs.

List of Key Publications:

1. Ferraiuolo, M.A., Sanyal, A., Naumova, N., Dekker, J., and Dostie J. (2012) From cells to chromatin: capturing snapshots of genome organization with 5C technology. *Methods 58*(3): 255-67. PubMed ID: 23137922

2. Fraser, J., Ethier, S.D., Miura, H., and Dostie, J. (2012) A Torrent of data: mapping chromatin organization using 5C and high-throughput sequencing. *Methods in Enzymology 513*: 113-141. PubMed ID: 22929767

3. Ferraiuolo, M.A., Rousseau, M., Miyamoto, C., Shenker, S., Wang, X.Q.D., Nadler, M., Blanchette, M., and Dostie, J. (2010) The three-dimensional architecture of Hox cluster silencing. *Nucleic Acids Research 38*(21): 7472-7484. PubMed ID: 20660483

4. Crutchley, J.L., Wang, X.Q.D., Ferraiuolo, M.A., and Dostie, J. (2010) Chromatin conformation signatures: ideal human disease biomarkers? *Biomarkers in Medicine 4(4),* 611-629. PubMed ID: 20701449

5. The FANTOM consortium and RIKEN Omics Science Center (2009). The transcriptional network that controls growth arrest and differentiation in a human myeloid leukemia cell line. *Nature Genetics* 41(5): 553-562. PubMed ID: 19377474

Circulating microRNAs as a lung tumour proxy: Determining whether a small RNA species in serum can be used as an early cancer detection tool

Terry Fox New Investigator Operating Grant (2010-2013)

Investigator: Cathie Garnis, University of British Columbia **Collaborator:** Stephen Lam, BC Cancer Agency

Scientific Summary: Lung cancer (LC) is the world's leading cause of cancer death. To improve outcomes, new methods for early disease detection are needed as this will facilitate intervention when tumours are at earlier, more treatable stages. Recently, microRNAs (miRNAs) circulating in blood were found to have utility as markers for the detection of many cancer types (including LC). However, the application of miRNA markers as a means for detecting pre-cancer or for assessing tumour progression likelihood has not been assessed. Further, detection sensitivity for miRNA markers has not been evaluated against established LC-screening methods.

Objectives:

- Identify LC-associated miRNAs circulating in blood that can be used as adjuncts for disease detection. This will first be achieved by subtractive analysis of blood samples obtained simultaneously from the same patient. This is a novel approach based on intra-individual controls; miRNA levels in pulmonary venous effluent draining the tumour vascular bed will be compared to levels in matched systemic arterial blood. Analysis of miRNA profiles in peripheral venous blood samples taken before and after surgical resection will refine our candidate list.
- Evaluate the utility of these miRNAs as markers for early detection of LC and for differentiating benign and malignant lung nodules detected by low-dose spiral computed tomography. Any circulating miRNA markers we identify will also be analyzed in concert with other molecular and imaging results accrued under the TFRI-sponsored Early Detection of Lung Cancer: A Pan Canadian Study.

List of Key Publications:

1. MacLellan S, Lawson J, Baik J, Guillaud M, Poh C, Garnis C. (2012) Serum biomarkers for detection of oral cancer and precancer, *Cancer Medicine*, 1(2):268-74.

2. MacLellan S, MacAulay C, Lam S, Garnis C. (submitted Apr 2013). Pre-profiling factors influencing serum microRNA levels, *PLoS One*, submitted.

The impact of hypoxia on the maturation of extracellular proteins that regulate tumour metabolism and microenvironment

Terry Fox New Investigator Operating Grant (2011-2014)

Investigator: Marianne Koritzinsky, Princess Margaret Cancer Center **Collaborator:** Rob Bristow, UHN

Scientific Summary: Poorly oxygenated (hypoxic) tumour cells represent a major limitation to the success of current cancer therapy due to treatment resistance and their influence on several aspects of malignancy. Cancer cells respond to hypoxia by metabolic adaptation which includes induced expression of cell surface proteins that mature in the endoplasmic reticulum (ER) following synthesis. We have recently shown that hypoxia results in severe ER stress due to a specific defect in post-translational disulfide bond isomerization in ER cargo proteins. Since many cargo proteins depend on disulfide isomerization, this defect results in suppression of overall surface protein expression. In contrast, some trans-membrane nutrient and metabolite transporters are efficiently expressed under hypoxia, suggesting that hypoxia-induced proteins destined for cell surface expression have a superior ability to mature and fold in the absence of oxygen. We hence hypothesize that ER-localized protein maturation is an important determinant of metabolic adaptation to hypoxia and the tumour microenvironment.

Three aims are designed to address this hypothesis:

- Determine the oxygen-dependency of protein maturation in the ER and its importance for nutrient transporters.
- Identify components of the ER maturation machinery that support hypoxia-specific expression of cell surface proteins.
- Assess the importance of oxygen dependent protein maturation for tumour metabolism and microenvironment.

Impact: Through understanding a fundamental component of functional gene expression during hypoxia, this study will provide novel hypoxia biomarkers and new molecular targets for cancer therapy.

List of Key Publications:

1. Koritzinsky M*, Levitin F, van den Beucken T, Harding N, Chu K, Boutros P, Braakman I, Wouters BG. Two phases of disulfide bond formation with differing requirements for oxygen. In minor revision (2013).
2. Koritzinsky M*, Wouters BG. The roles of reactive oxygen species and autophagy in mediating the tolerance of Tumour cells to cycling hypoxia. *Seminars in Radiation Oncology*: Oct; 23 (4): 252-261 (2013).
3. Cojocari D, Vellanki RN, Sit B, Uehling D, Koritzinsky M, Wouters BG. New small molecule inhibitors of UPR activation demonstrate that PERK, but not IRE1α signaling is essential for promoting adaptation and survival to hypoxia. *Radiotherapy and Oncology:* (2013) [epub ahead of print].
4. Rouschop KM, Dubois LJ, Keulers TG, van den Beucken T, Lambin P, Bussink J, van der Kogel AJ, Koritzinsky M, Wouters BG. PERK/eIF2α signaling protects therapy resistant hypoxic cells through induction of glutathione synthesis and protection against ROS. Proceedings of the National Academy of Sciences U S A: Mar 19;110(12):4622-7 (2013).
5. Rouschop KM, van den Beucken T, Dubois L, Niessen H, Bussink J, Savelkouls K, Keulers T, Mujcic H, Landuyt W, Voncken JW, Lambin P, van der Kogel AJ, Koritzinsky M, Wouters BG. The unfolded protein response protects human Tumour cells during hypoxia through regulation of the autophagy genes MAP1LC3B and ATG5. *Journal of Clinical Investigations:* Jan; 120(1):127-41 (2010).
6. Wouters BG, Koritzinsky M. Hypoxia signaling through mTOR and the unfolded protein response in cancer. *Nature Reviews Cancer:* 8(11):851-64 (2008).
7. Koritzinsky M, Rouschop KR, Magagnin MG, van den Beucken T, Savelkouls K, Wouters BG. Phosphorylation of eIF2α is required for mRNA translation inhibition and survival during moderate hypoxia. *Radiotherapy and Oncology*: Jun; 83(3):353-61 (2007).
8. Koritzinsky M, Magagnin MG, van den Beucken T, Savelkouls K, Koumenis C, Dostie J, Pyronnet S, Kaufman RJ, Weppler SA, Voncken JW, Lambin P, Sonenberg N, Wouters BG. Gene expression during acute and chronic hypoxia is mediated by distinct modes of translational control. *EMBO Journal:* Mar 8; 25(5):1114-25 (2006).
9. Bi M, Naczki C, Koritzinsky M, Fels D, Blais J, Hu N, Harding H, Novoa I, Varia M, Raleigh J, Scheuner D, Kaufman RJ, Bell J, Ron D, Wouters BG, Koumenis C. ER stress-regulated translation increases tolerance to extreme hypoxia and promotes tumour growth. *EMBO Journal:* Oct 5; 24(19):3470-81 (2005).
10. Koumenis C, Naczki C, Koritzinsky M, Rastani S, Diehl A, Sonenberg N, Koromilas A, Wouters BG. Regulation of protein synthesis by hypoxia via activation of the endoplasmic reticulum kinase PERK and phosphorylation of the translation initiation factor eIF2alpha. *Molecular and Cellular Biology:* Nov; 22(21):7405-16 (2002).

Role of BAF chromatin remodeling complexes in normal and leukemic hemopoiesis

Terry Fox New Investigator Operating Grant (2009-2013)

Investigator: Julie Lessard; Institute for Research in Immunology and Cancer, Université de Montréal

Scientific Summary: Mammalian hemopoietic stem cells (HSCs) have the capacity to both self-renew and generate all the myeloid and lymphoid cell types of the adult hemopoietic system. One major determinant of hemopoietic cell fates are patterns of chromatinization of genetic loci that are established during differentiation and that limit the possible outcomes of general signalling events to the activation of lineage-specific genes. Recent evidence suggests that specialized assemblies of SWI/SNF-like ATP-dependent BAF chromatin remodeling complexes are essential for *e*mbryonic *s*tem cell (esBAF) and *n*eural *p*rogenitor (npBAF) cell function. The main objective of this proposal is to define the biochemical nature and developmental roles of a novel family of *h*emopoietic hBAF chromatin remodelling complexes in normal and leukemic hemopoiesis.

More specifically, we propose:

- To address the potential roles of BAF45a and BAF53a (and eventually other novel stem cell-specific *h*BAF sub-units identified below) in regulating the generation, self-renewal and/or proliferation of normal and leukemia stem cells. These studies will involve the generation of conditional targeted *(floxed)* alleles of the *BAF45a* and *BAF53a* genes in the mouse, which are highly expressed in primitive subsets of mouse bone-marrow cells.
- To use state-of-the-art proteomics technologies to define the sub-unit composition and post-translational modifications of a novel family of *h*BAF chromatin remodelling complexes in normal and leukemic HSCs using:
    - HSC-enriched populations expanded *in vitro* using agonists of self-renewal.
    - Leukemia that significantly differ in their *in vivo* proliferative potential and in their frequency of leukemia-initiating cells (L-ICs), but are otherwise very similar morphologically, phenotypically and in their gene expression profiles (as determined by *Affymetrix* arrays).

List of Key Publications:

1. Krasteva V, Buscarlet M, Diaz-Tellez A, Bernard MA, Crabtree GR and Lessard JA. The BAF53a subunit of SWI/SNF-like BAF complexes is essential for hemopoietic stem cell function. *Blood*:120(24):4720-32 (2012).

2. Lessard J and Crabtree GR. Chromatin Regulatory Mechanisms in Pluripotency. *Ann. Rev. Cell. Dev. Biol:* 26:503-32 (Volume publication date November 2010).

3. Wu J, Lessard J, and Crabtree GR. Understanding the Words of Chromatin Regulation. Review Article. *Cell:* 136(2):200-6 (2009).

4. Lessard J*, Wu JI*, Olave IA, Qiu Z, Ghosh A, Graef IA. and Crabtree GR. Regulation of Dendritic Development by Neuron-Specific Chromatin Remodeling Complexes. *Neuron:* 56 (1): 94-108 (2007). (* Co-first authors.)

5. Lessard J*, Wu JI*, Ranish JA, Wan M, Winslow MM, Staahl BT, Wu H, Aebersold R, Graef IA. and Crabtree GR. An Essential Switch in Subunit Composition of a Chromatin Remodeling Complex during Neural Development. *Neuron:* 55(2): 201-15 (2007). (* Co-first authors.)

Randomized controlled trial comparing colposcopy to HPV testing to identify persistent or recurrent high-grade cervical cancer precursors (CoHIPP)

Terry Fox New Investigator Operating Grant (2009-2014)

Investigators: Marie-Hélène Mayrand, CRCHUM; Michal Abrahamowicz, McGill University Health Center; James Bentley, Dalhousie University; François Coutlée, UdeM; Helen Trottier, Hôpital Ste-Justine; Paul Bessette, Center hospitalier universitaire de Sherbrooke; Thomas G Ehlen, BC Cancer Agency; Lorraine Margaret Elit, Juravinski Cancer Center, Hamilton; Susie Lau, Jewish General Hospital, Montréal; Marie Plante, Center hospitalier affilié universitaire de Québec; Philippe Sauthier, Center hospitalier universitaire de Montréal and Bernard Têtu, Hôtel-Dieu de Québec.

Scientific Summary: Cervical cancer ranks 13th in terms of cancer incidence in Canadian women but remains the second most frequent cancer in Canadian women aged 20 to 44. Fortunately, its natural history makes it an ideal candidate for successful prevention. Cervical cancer and its precursors are caused by a persistent infection of the cervical epithelium by one of the 12 oncogenic (or high risk/HR) types of human papillomaviruses (HPVs). The pre-invasive changes of cervical intra-epithelial neoplasia (CIN) and adenocarcinoma *in situ* (AIS) can be identified through cervical cytology (also known as the Pap test). When cellular abnormalities are identified, women are referred for diagnostic testing. High-grade precursor lesions (HG-CIN/AIS) carry a high risk of progression to invasive cancer and for this reason their treatment is recommended. Treatment success rates of HG-CIN/AIS are estimated at 85%. However, those who fail treatment need to be identified promptly because their risk of invasive disease is significantly increased. The current strategy used in Canada to identify treatment failures consists of a follow-up every six months in colposcopy clinics for two years. Recent research underlines the fact that routine colposcopy is unreliable and may miss significant lesions. The identification of HR-HPV DNA in the cervical secretions is known as HPV testing. A few studies have investigated the use of HPV testing post-treatment to identify of treatment failures. Although they point to a very good sensitivity (90-95%), there were methodological limitations precluding firm conclusions: studies were small; the HPV test used was often not suitable for clinical laboratory use; endpoints were not assessed by histological confirmation. Most importantly, none compared HPV testing to colposcopy, the strategy used in Canada.

Research Question: Is HPV testing more accurate than routine colposcopy to identify CIN2/3 treatment failures?

Methodology: We are conducting a parallel, randomized controlled trial, where participants (women treated for high-grade precursor lesions) are randomized 1:1 to routine colposcopy versus HPV testing after treatment of CIN2/3 lesions six months after treatment. All participants will undergo expert diagnostic assessment at 12 and 24 months. The sample size was calculated to have 80% power to detect an increase in sensitivity of 15%, with an alpha set at 0.05 (double sided test). Assuming a 15% failure rate of treatment and a 20% drop out rate, a total of 2,250 participants will need to be accrued. The primary analysis will consist of the comparison of the diagnostic indices of the two follow-up strategies: sensitivity, specificity, positive and negative predictive values (with their 95% asymptotic confidence intervals). Cumulative persistent/recurrent cases identified during the two year follow-up will make up the case group.

Progress: Recruitment was completed in March 2013. Over 95% of randomization visits have been completed, as planned in the timeline. We expect follow-up visits to be completed by spring 2015. The design of the trial was presented at international venues and the results are very much awaited by the international community. Supplementary funding was secured through CIHR in March 2013 to address ancillary research questions.

Expected Contribution: The result from this trial will provide high-quality data on which to base management recommendations. If HPV testing is found to be more accurate than routine colposcopy, follow-up of treated HG-CIN may occur mainly with primary health care providers with HPV testing, reducing costs, and making it possible to focus colposcopy activities on a group of women truly at risk of significant disease.

Epithelial polarity in tumour invasion and metastasis

Terry Fox New Investigator Operating Grant (2011-2014)

Investigator: Luke McCaffrey, McGill University **Collaborator:** William Muller, McGill University

Scientific Summary: Under the mentorship of the Preclinical Models and Therapeutic Targets for Metastatic Breast Cancer Program, our objective is to build a molecular understanding of how cell polarity regulates invasive and metastatic breast cancer with the goal of identifying novel prognostic markers and therapeutic targets. Metastatic progression correlates strongly with loss of tissue structure and organization; accordingly, cell polarity proteins are frequently disrupted in tumours, but the role of these key regulators in breast cancer progression is not understood. Accumulating evidence indicates that atypical protein kinase C (aPKC) isoforms, which are key transducers of polarity signalling, are disrupted in breast cancer and may have either oncogenic or tumour suppressive functions. Furthermore, we have found that the Par3 polarity protein is a key regulatory of aPKC in normal and tumourigenic epithelial cells. In this project we are further exploring the contribution of Par3 and aPKC in breast tumour progression. To address the objectives of our project, we have three primary questions:

- What are the mechanisms by which Par3/aPKC polarity organizes the mammary epithelium, and how this is disrupted in breast cancer?
- How do aPKC isoforms promote or suppress breast cancer progression?
- Does aPKC polarity mediate cancer stem cell activity to control the differentiation state of breast tumours?

Key Findings: We found that Par3 silencing dramatically reduced tumour latency in breast cancer mouse models and produced invasive and metastatic tumours that retained epithelial marker expression. Par3 depletion was associated with induction of matrix metalloproteinases, destruction of the extracellular matrix, and invasion, all mediated by atypical PKC-dependant JAK/Stat3 activation. Importantly, we found that Par3 expression is significantly reduced in human breast cancers, which correlates with active aPKC and Stat3. These data identify Par3 and aPKC as regulators of signaling pathways relevant to invasive breast cancer.

List of Key Publications:

1. McCaffrey L, Macara IG. The Par3/aPKC interaction is essential for end bud remodelling and progenitor differentiation during mammary gland morphogenesis. *Genes Dev:* 23(12) 1450-60 (2009).

2. McCaffrey L, Macara IG. Widely conserved signalling pathways in the establishment of cell polarity. *Cold Spring Harb Perspect Biol*: 1(2) 1-17 (2009).

3. McCaffrey L, Macara IG. Epithelial organization, cell polarity, and Tumourigenesis. *Trends Cell Biol*: 21(12) 625-680 (2011).

4. McCaffrey L, Montalbano J, Mihai C, Macara IG. Loss of the Par3 Polarity Protein Promotes Breast Tumourigenesis and Metastasis. *Cancer Cell:* 22, 601-614 (2012).

5. McCaffrey L, Macara IG. Signaling Pathways in Cell Polarity. *Cold Spring Harb Perspect Biol*. 4(6): 1-15 (2012).

6. Macara IG, McCaffrey L. Cell Polarity in Morphogenesis and Metastasis. *Phil Trans R Soc B* (In Press).

The role of Cep192 in centrosome biogenesis

Terry Fox New Investigator Operating Grant (2008-2013)

Investigator: Laurence Pelletier, Mt. Sinai Hospital

Scientific Summary: A crucial step during cellular division is the accurate segregation of chromosomes to progeny. This is accomplished by an elaborate bipolar microtubule-based structure called the mitotic spindle. At the heart of mitotic spindle assembly are centrosomes. Centrosomes are composed of a centriole pair and microtubule nucleating material called pericentriolar material. In order for a stable bipolar mitotic spindle apparatus to form, centrosomes need to duplicate once and only once during the cell cycle, a process regulated through the tight control of centriole duplication. Centriole duplication and assembly play a crucial role in spindle formation by organizing and defining the number of spindle poles. Defects in centriole biogenesis can therefore lead to mono-/multi-polar spindles and cause aneuploidy, a condition associated with cancer formation. The original grant application was rooted in the overall goal to better understand, at the molecular level, proteins required for mitotic spindle assembly, in particular the role of the centrosome protein Cep192 in the regulation of centriole duplication and centrosome maturation.

The three specific aims of this research are:

- Defining the role of Cep192 in centriole duplication.
- The regulation and dynamics of Cep192 during the cell cycle.
- What targets Cep192 to centrosomes?

List of Key Publications:

1. *Kittler R, *Pelletier L, Heninger AK, Slabicki M, Theis M, Miroslaw L, Poser I, Lawo S, Grabner H, Kozak K, Wagner J, Surendranath V, Richter C, Bowen W, Jackson AL, Habermann B, Hyman AA, Buchholz F. Genome-scale RNAi profiling of cell division in human tissue culture cells. *Nat Cell Biol*: Dec;9(12):1401-12 (2007).*Joint-first authors.

2. Zhu F, Lawo S, Bird A, Pinchev D, Ralph A, Richter C, Müller-Reicher T, Kittler R, Hyman A, Pelletier L. The mammalian SPD-2 ortholog Cep192 regulates centrosome biogenesis. *Curr Biol*: Jan 22;18(2):136-41 (2008).

3. Lawo S, Bashkurov M, Mullin M, Ferreria MG, Kittler R, Habermann B, Tagliaferro A, Poser I, Hutchins JR, Hegemann B, Pinchev D, Buchholz F, Peters JM, Hyman AA, Gingras AC, Pelletier L. HAUS, the 8-Subunit Human Augmin Complex, Regulates Centrosome and Spindle Integrity. *Curr Biol:* May 26;19(10):816-26 (2009)

4. Hutchins JR, Toyoda Y, Hegemann B, Poser I, Hériché JK, Sykora MM, Augsburg M, Hudecz O, Buschhorn BA, Bulkescher J, Conrad C, Comartin D, Schleiffer A, Sarov M, Pozniakovsky A, Slabicki MM, Schloissnig S, Steinmacher I, Leuschner M, Ssykor A, Lawo S, Pelletier L, Stark H, Nasmyth K, Ellenberg J, Durbin R, Buchholz F, Mechtler K, Hyman AA, Peters JM. Systematic analysis of human protein complexes identifies chromosome segregation proteins. *Science:* Apr 30;328(5978):593-9 (2010).

5. Lawo, S., Hasegan, M and Pelletier, L. Sub-diffraction imaging reveals higher-order organization and assembly principles of pericentriolar material. *Nature Cell Biology* 2012

6. Gomez-Ferreria, MA., Bashkurov, M., Helbig, AO., Larsen, B., Pawson, T., Gingras, AC and Pelletier, L. Novel NEDD1 phosphorylation sites regulate γ-tubulin binding and mitotic spindle assembly. *Journal of Cell Science* 2012

7. Gomez-Ferreria, M.A., Bashkurov, M., Mullin, M., Gingras, A.C., and Pelletier, L. (2012). CEP192 interacts physically and functionally with the K63-deubiquitinase CYLD to promote mitotic spindle assembly. *Cell cycle*. 2012

Are genomic instability and clonal diversity prognostic indicators of high-grade serous ovarian cancer?

Terry Fox New Investigator Operating Grant (2012-2015)

Investigator: Sohrab Shah, BC Cancer Agency **Collaborator:** David Huntsman, BC Cancer Agency

Scientific Summary: In North America, ovarian cancer is the leading cause of death due to gynecological malignancies. The majority of women diagnosed with this disease are not expected to survive beyond five years. This project focuses on the most common subtype, high-grade serous ovarian cancers which account for 70% of ovarian cancers. The current standard of treatment involves platinum-based chemotherapies, however, though they are effective in treating the primary tumour, in almost all instances, the cancer will reform and are resistant to any of the currently available therapies. Current clinical markers are ineffective in determining which patients will respond more effectively to chemotherapy and live longer without evidence of disease. High-grade serous carcinomas are genomically diverse and heterogeneous, i.e., their genetic make-up vary from tumour to tumour and within the same tumour, populations of cells can be very different from one another.

Our research group recently observed that there are, in fact, global patterns of diversity that exist amongst this group of tumours. This proposal will examine whether these global patterns of genomic diversity can be used to segregate patient populations and predict which patients fare better than others. We have assembled a highly productive and multidisciplinary team to undertake this research. State-of-the-art genome sequencing technology and novel bioinformatic and algorithmic approaches developed in our lab will be used to decipher the entire DNA sequence of the tumours which will be compared to the patient's normal DNA to identify global patterns of change. In addition, using state-of-the art single-cell microfluidic devices developed by our group, we will be able to sequence the DNA in individual cells to determine whether there are subpopulations of cells within the primary tumour that are resistant to platinum-based therapy and emerge as dominant clones in relapsed patients. All the tools, infrastructure and tissue specimens required for the discovery phase of this project are available within the BC Cancer Agency and UBC.

We will validate our findings in a much larger patient population (>500 samples) using a national ovarian cancer resource (COEUR) funded by the Terry Fox Research Institute. The ultimate goal is to develop biomarkers or parameters that can be used clinically to predict a patient's response to chemotherapy. The discoveries from this research can be immediately translatable to other cancer types that follow similar patterns of evolution and progression.

The role of Bmi1 in neural stem cell maintenance and brain tumour development

Terry Fox New Investigator Operating Grant (2011-2014)

Investigator: Sheila Singh, McMaster University

Collaborators: Gary Bader, UofT; John Hassell, McMaster University; David Kaplan, UofT; Robert Rottapel, OCI ,UofT

Scientific Summary: Glioblastoma (GBM), the most common primary brain tumour in adults, is one of the most aggressive human cancers, feared for its near uniformly fatal prognosis. We previously isolated and characterized a minority subpopulation of GBM cells with stem cell properties that were exclusively capable of driving brain tumour formation in a human-mouse xenograft model. The discovery of the BTIC (brain tumour initiating cell) reinforces the concept that common molecular pathways may govern both brain development and the growth of a brain tumour. This research proposal will examine the role of the master regulatory neural stem cell (NSC) gene Bmi1 in the initiation and maintenance of brain tumours.

Research aims include:

- Knockdown of Bmi1 in human GBM BTICs and injection of cells into mouse brains, utilizing our BTIC xenograft model, to elucidate the role of Bmi1 in brain tumour development.
- Transduction of a lentiviral construct overexpressing Bmi1 into primary NSCs and subsequent injection of cells into mouse brains, to determine if gain of Bmi1 is necessary and sufficient to induce tumour formation.
- Performance of microarray experiments and RNA-sequencing on human NSCs and GBM BTICs, and comparison of gene expression data with occupancy data generated from promoter tiling arrays and ChIP-sequencing for Bmi1, to elucidate what genes Bmi1 differentially binds to in normal NSCs and in GBM BTICs.
- We aim to map a comprehensive gene network governed by Bmi1 which will reveal its function in BTICs and in brain tumourigenesis, providing future selective therapeutic targets for children and adults with brain tumours.

List of Key Publications:

1. Singh, S.K., *et al.* Identification of human brain tumour initiating cells. *Nature* 432, 396-401 (2004).
2. Facchino, S., Abdouh, M., Chatoo, W. & Bernier, G. BMI1 confers radioresistance to normal and cancerous neural stem cells through recruitment of the DNA damage response machinery. *J Neurosci* 30, 10096-10111 (2010).
3. Sauvageau, M. & Sauvageau, G. Polycomb group proteins: multi-faceted regulators of somatic stem cells and cancer. *Cell stem cell* 7, 299-313 (2010).
4. X Wang, C Venugopal, B Manoranjan, N McFarlane, E O'Farrell, S Nolte, C Hawkins, T Gunnarsson, R Hollenberg, J Kwiecien, and SK Singh. Sonic hedgehog directly regulates Bmi1 in human medulloblastoma brain tumour initiating cells. *Oncogene*: 2012 Jan 12;31(2):187-99.
5. A Fotovati, S Abu-Ali, PS Wang, C Lee, JY Chen, S Franciosi, J Triscott, Y Nakamura, Y Sugita, T Uchiumi, M Kuwamo, BR Leavitt, SK Singh, B Reynolds, A Jury, C Jones, CJ Pallen, SE Dunn. YB-1 is a key factor in normal brain development and contributes to gliomagenesis through the maintenance of neural stem cells. *Cancer Research* 2011: 71(16): 5569-78.
6. N Li, C Venugopal, X Wang, B Manoranjan, M Lenkiewicz, T Gunnarsson, R Hollenberg, P Klurfan, N Murty, C Wynder and SK Singh. Bmi1 marks intermediate precursors during differentiation of human brain Tumour initiating cells. *Stem Cell Research* 2012: 8; pp 141-153.
7. B Manoranjan, C Venugopal, N McFarlane, B Doble, SE Dunn, K Scheinemann, SK Singh. Medulloblastoma stem cells: where development and cancer cross pathways. *Pediatric Research*; 2012 Apr;71(4 Pt 2):516-22.24.
8. B Manoranjan, C Venugopal, SE Dunn, K Scheinemann, B Doble, SK Singh. Medulloblastoma Stem Cells. *CANCER LETTERS* 2012 Jul 14. [Epub ahead of print].
9. C Venugopal, N McFarlane, SM Nolte, B Manoranjan, SK Singh. Processing of primary brain Tumour tissue for stem cell assays and flow sorting. *Journal of Visual Experimentation* (JoVE) 2012 Sep 25;(67).
10. C Lee, Abbas Fotovati, Nichole McFarlane, Chitra Venugopal, Joanna Triscott, Ash Singhal, Christopher Dunham, Maite Verreault, Marcel Bally, Brent Reynolds, Stephen Yip, Hiroaki Wakimoto, Aru Narendran, Sheila K Singh, Sandra E Dunn. Polo-Like Kinase 1 (PLK1) Inhibition Kills Brain Cancer Cells and Suppresses Self-Renewal of Brain Tumour Initiating Cells. *Stem Cells*. 2012 Jun;30(6):1064-75.
11. C Venugopal, B Manoranjan, N McFarlane, E O'Farrell, S Nolte, X Wang, J Kwiecien, S Wang, M Siu, SK Singh. The GBM secretome induces transformation of human neural precursor cells. *J Neurooncol*. 2012 Sep;109(3):457-66.
12. SM Nolte, C Venugopal, N McFarlane, O Morozova, RM Hallett, E O'Farrell, B Manoranjan, NK Murty, E Kachur, P Klurfan, J Provias, JA Hassell, M Marra, SK Singh. A cancer stem cell model for studying brain metastases from primary lung cancer. *JNCI* (IF 14.9), J Natl Cancer Inst. 2013 Apr 17;105(8):551-62.
13. SK Singh, B Manoranjan, C Venugopal. Evolution of brain Tumour-initiating cell research: In pursuit of a moving target. *Future Neurology* 2013; 8(1): 1–3.
14. B Manoranjan, X Wang, R Hallett, C Venugopal, MD Taylor, J Hassell , K Scheinemann, J Provias, S Mak, F Farrokhyar, SE Dunn and SK Singh. FoxG1 interacts with Bmi1 to regulate self-renewal and Tumourigenicity of medulloblastoma stem cells. *Stem Cells*. 2013 Apr 17 doi 10.1002/stem.1401. [Epub ahead of print].
15. H Zarkoob, SK Singh, SA Mani, M Kohandel. Investigating the link between molecular subclasses of GBM, the epithelial-mesenchymal transition, and CD133+/- cells. *PLoS One* 2013 May 29;8(5):e64169. doi: 10.137

Exhaustion of tumour-initiating cells by targeting their self-renewal capacity with telomerase inhibitors

Terry Fox New Investigator Operating Grant (2010-2013)

Investigator: Uri Tabori, Hospital for Sick Children **Collaborator:** Rob Rottapel, UofT and OICR

Scientific Summary: Tumour recurrence is the major cause of death in paediatric cancer and is thought to result from persistence of tumour initiating cells (TIC) even after no residual disease is seen. We have revealed that telomerase is active only in the TIC subpopulation of paediatric neural tumours and not in the bulk of tumour cells or corresponding normal tissue stem cells. Telomerase inhibition causes irreversible loss of self renewal and stem cell capacities of paediatric neural TIC and does not affect normal stem cells. We will develop novel therapeutic approaches for paediatric cancers through the following specific aims:

- To determine the mechanisms and pathways governing the irreversible loss of stem cell capacity of TIC in paediatric neuroblastoma.
- Confirm our preclinical data in humans by examining normal and malignant stem cell capacities in patients undergoing Phase I trial with our telomerase inhibitor.
- Develop novel therapies for neuroblastoma using telomere-based TIC exhaustion.

Upon completion of this project, we will open a new window to the mechanisms underlying relapse in cancer and will provide novel and safe therapies for children with malignant neural tumours.

Clinical Trial: A trans-Canadian Phase I clinical trial based on our results on exhaustion of neuroblastoma stem cells by telomerase inhibition with Imetelstat and retinoic acid is opening. My laboratory is the central lab for this trial.

List of Key Publications:

1. Pedro Castelo-Branco, Sanaa Choufani, Stephen Mack, Denis Gallagher, Cindy Zhang, Tatiana Lipman, Nataliya Zhukova, Erin J Walker, Dianna Martin, Diana Merino, Jonathan D. Wasserman, Cynthia Elizabeth, Noa Alon, Libo Zhang, Volker Hovestadt, Marcel Kool, David T.W. Jones, Sidney Croul, Cynthia Hawkins, Johann Hitzler, Jean C.Y. Wang, David Malkin, Sylvain Baruchel, Peter B. Dirks, Stefan Pfister, Michael D. Taylor, Rosanna Weksberg and Uri Tabori. hTERT promoter hypermethylation is a cancer signature which predicts survival and response to targeted therapy in pediatric nervous system Tumours. *Lancet Oncol* 2013.

Role of targeted phosphatase activity in regulation of cell proliferation

Terry Fox New Investigator Operating Grant (2009-2014)

Investigator: Laura Trinkle-Mulcahy, University of Ottawa

Scientific Summary: The majority of molecular events that take place in the cell are modulated by reversible protein phosphorylation events, and it is thus not surprising that aberrant phosphorylation signalling pathways have been linked to tumour initiation and progression in numerous types of cancer. Our laboratory focuses on the identification and characterization of protein phosphatase regulatory proteins, as they offer the specificity and thus the potential for targeted therapeutic strategies that catalytic site inhibitors do not. Using a powerful combination of live-cell fluorescence imaging of tagged protein phosphatase 1 (PP1) and quantitative mass spectrometry-based proteomics, we have assembled a map of the diverse multiprotein complexes to which this enzyme is dynamically targeted throughout the cell. Having previously identified the essential RepoMan/PP1 complex, which has since been linked to regulation of mitotic exit, DNA damage response pathways and programmed cell death, we turned our attention to identification of its substrates in these specific pathways. We adapted our powerful imaging/proteomics combination in a unique "fragmentome" approach, first demonstrating distinct localization patterns and turnover dynamics for specific domains within the protein (which reflect underlying binding events) and then mapping their interactomes independently. By reducing the complexity of the experiment (i.e. focusing on specific binding domains of this large, modular protein), we significantly increased the sensitivity of detection of low abundance/low affinity interactors above background, identifying a wide range of previously unknown interactors that point to novel roles for the RepoMan/PP1 complex in mitosis, ribosomal protein import and DNA damage repair. With the goal of disrupting the pro-survival function of RepoMan, we mapped the binding domains further to determine the minimal amino acid sequence mediating association with DNA damage-related substrates. We are now testing whether overexpression of these dominant-negative mutants increases the sensitivity of cancer cells to treatment with DNA damage-inducing agents by displacing endogenous RepoMan/PP1 complexes. It is our hope that molecular mimetics of these mutants could one day be employed in cancer therapy approaches as highly specific phosphatase inhibitors, with little or no off-target effects.

Specific Aims:

- Identify and functionally characterize interphase and mitotic RepoMan/PP1 complexes.
- Explore the mechanism of targeting of RepoMan/PP1 to chromatin.
- Examine the mechanism by which displacement of endogenous RepoMan/PP1 complexes triggers apoptosis.

List of Key Publications:

1. Prévost, M., Chamousset, D., Nasa, I., Freele, E., Morrice, N., Moorhead, G., Trinkle-Mulcahy, L. Quantitative fragmentome mapping reveals novel, domain-specific partners for the modular protein RepoMan. *Mol Cell Proteomics* 12:1468-86, 2013.

2. Trinkle-Mulcahy L. Resolving protein interactions and complexes by affinity purification followed by label-based quantitative mass spectrometry. *Proteomics*.12:1623-38, 2012.

3. Taperin (c9orf75), a mutated gene in nonsyndromic deafness, encodes a vertebrate specific, nuclear localized protein phosphatase one alpha (PP1α) docking protein. Ferrar T, Chamousset D, De Wever V, Nimick M, Andersen J, Trinkle-Mulcahy L, Moorhead GB. *Biol Open*. 2012 Feb 15;1(2):128-39.

4. Chamousset D, DeWever V, Moorhead G, Chen Y, Boisvert FM, Lamond AK and Trinkle-Mulcahy L. RRP1B targets PP1 to mammalian cell nucleoli and is associated with pre-60S ribosomal subunits. *Mol Biol Cell*: 21:4212-26 (2010).

5. Chamousset D, Mamane S, Boisvert FM, Trinkle-Mulcahy L. Efficient extraction of nucleolar proteins for interactome analyses. *Proteomics*: 10:3045-50 (2010).

6. Trinkle-Mulcahy L, Boulon S, Lam YW, Urcia R, Boisvert FM, Vandermoere F, Morrice NA, Swift S, Rothbauer U, Leonhardt H and Lamond AI. Identifying specific protein interaction partners using quantitative mass spectrometry and bead proteomes. *J. Cell Biol:* 183:223-39 (2008).

7. Trinkle-Mulcahy L, Andersen J, Lam YW, Moorhead G, Mann M and Lamond AI. Repo-Man recruits PP1γ to chromatin and is essential for cell viability. *J. Cell Bio:* 172:679-92 (2006).

Exploring novel mechanisms of tumour vascularization in malignant brain tumours

Terry Fox New Investigator Operating Grant (2012-2015)

Investigator: Gelareh Zadeh, UHN **Collaborator:** Andras Nagy, Mt. Sinai Hospital

Scientific Summary: Angiogenesis involves a highly regulated and co-ordinated interaction of multiple angiogenic factors and is critical for both embryonal development and physiological vessel formation in adults. Angiogenesis is also proven to significantly contribute to the progression of various disease processes, including cancer.

Glioblastoma (GBM) is among the most angiogenic tumors, and it therefore makes sense from an investigative perspective to understand the mechanisms of angiogenesis in these tumours as a means for identifying new therapeutic opportunities. However, despite initial positive early response to anti-angiogenic therapy in GBMs, the clinical benefits of anti-angiogenic treatment is limited and GBM recurrence within a few months remains inevitable. This is, in part, due to unidentified mechanisms of neo-vascularization that can evade radiation and targeted therapy. Therefore, recent research interest has focused on the possibility of new vessel formation through progenitor cells that are replenished at a constant and continuous level and as a result they can avoid therapeutics.

Neovascularization has traditionally been considered to occur through two distinct processes, angiogenesis and vasculogenesis. Angiogenesis is the process by which new vessels form from sprouting and branching of pre-existing vessels, whereas vasculogenesis or *de novo* vessel formation, occurs by differentiation of endothelial precursor cells (EPC). It has long been thought that vasculogenesis was restricted to embryonal vessel development while post-natal vessel formation occurs primarily through angiogenesis. However, emerging evidence suggests that vasculogenesis can occur in adult life and it has been argued to provide a potential mechanism for cancer neovascularization through mobilization of EPCs from the bone marrow (BM) or circulation. This hypothesis remains highly controversial and there is debate as to whether bone marrow derived progenitor cells (BMDCs) actually differentiate to endothelial cells (ECs) or contribute to formation of vascular channels in neoplastic processes.

Additional open questions include whether BMDC vasculogenesis is influenced by tumor type, microenvironmental factors, tumour growth stage and response to therapy. To do this, however, we needed a model that could examine the process of vasculogenesis *in vivo*, and in real time. This is the impetus for us to establish the experimental strategies outlined above in (Ai), using a two-photon laser microscopy (2PLM) system, coupled with an intra-cranial window in mouse models of GBM to obtain *in-vivo* real-time longitudinal imaging of normal brain and GBM associated vasculature. This strategy complements traditional immunohistochemical (IHC) and immunofluorescent (IF) analysis, as it allows visualization at single cell resolution of fluorescent bone marrow derived progenitor cells, and fluorescent GBM cells. It then goes beyond the traditional IHC and IF techniques by the examination of these processes in real time, in a living mammalian organism. In a series of carefully controlled experiments, we observed that bone marrow-derived cells (BMDCs) are recruited to the brain in response to cranial radiation (CR).

We demonstrated that BMDCs are recruited specifically to the site of CR, in a radiation dose and temporal-spatial manner. We showed that BMDCs do not form endothelial cells but rather they differentiate predominantly into inflammatory cells and microglia. Using a GBM *in vivo* model, we show three distinct patterns of BDMC activity in response to tumour growth. While these cells do support GBM neo-vascularization, we definitively show that there is no evidence of direct differentiation of BMDCs into endothelial cells and, moreover, no contribution to vasculogenesis or to *de novo* vessel formation. This result is in contrast to prior studies by other groups, and we strongly believe that the careful use of our 2PLM high-resolution *in vivo* optical imaging single-cell resolution system in real time was critical in resolving this controversial topic in the field of GBM angiogenesis. Additionally, these findings support the concept that a disruption of the region-dependent contribution of BMDCs to vasculogenesis may be an important therapeutic opportunity.

Defining the role of lyn tyrosine kinase in prostate cancer progression to castrate resistant stage

Terry Fox New Investigator Operating Grant (2010-2013)

Investigator: Amina Zoubeidi, Vancouver Prostate Centre (VPC), UBC **Collaborator:** Paul Rennie, VPC, UBC

Scientific Summary: The objective is to evaluate the importance of the non receptor Lyn tyrosine kinase in PCa progression, and thus as a likely therapeutic target.

Background: The process by which PCa inevitably progresses to its terminal, castration-resistant stage, is most likely dependent on tyrosine kinases. Genetic manipulation of several tyrosine kinases *in vivo* revealed that only a knockout of Lyn, compromised prostate gland development. Our own preliminary data showed that targeting Lyn kinase abrogates AR transcriptional activity and decreases cell proliferation. We therefore hypothesize that Lyn tyrosine kinase enhances the androgen receptor (AR) transcriptional activity and promotes the progression of PCa under hormonal withdrawal to the castrate resistant stage. We are using molecular and functional analyses (gain and loss of Lyn functional studies) to show how Lyn kinase modulates AR activation. We are working on elucidating the role of Lyn expression and activation in PCa progression using a xenograft PCa tumour model and a transgenic mouse model. We are currently determining the profiles of both Lyn expression and activation in prostate tumours. We validate our finding on our human PCa tissue microarray (TMA) containing cores from naive prostatectomy specimens and specimens after neo-adjuvant hormonal withdrawal as well as biopsies from CRPC and found that Lyn expression and activation correlate with aggressive phenotype and CRPC compared to naïve prostate cancer patient specimens. Overexpression of Lyn drives CRPC in a LNCaP xenograft model and activates AR both *in vitro* and *in vivo*. Specific targeting of Lyn tyrosine kinase by siRNA resulted in a decrease of AR transcriptional activity by inhibiting AR nuclear translocation. Lyn knockdown decreases the association of AR and heat shock protein 90 which, in turn, induces proteasome-mediated AR degradation. This destabilization of the AR is associated with a concomitant decrease of cell proliferation and induction of apoptosis. This work has identified a novel mechanism of AR transcriptional activity and expression regulation by the Lyn tyrosine kinase, specifically in CRPC. Importantly, our results justify further investigation of Lyn tyrosine kinase as a therapeutic target for the treatment of CRPC. Recently we found that Lyn is not only critical for progression to CRPC but is required for epithelial mesenchymal transition by stabilizing SLUG and could be considered as a therapeutic target not only in CRPC but also in metastatic setting.

List of Key Publications:

1. Shiota M, Bishop JL, Nip K, Zardan A, Takeushi A, Cordonnier T, Beraldi E, Bazov J, Fazli L, Chi KN, Gleave M, Zoubeidi A. Hsp27 regulates epithelial mesenchymal transition, metastasis and circulating tumor cells in prostate cancer. *Cancer Res*. 2013 May 15;73(10):3109-19.

2. Kuruma H, Matsumoto H, Shiota M, Bishop JL, Lamoureux F, Thomas C, Briere D, Los G, Gleave M, Fanjul A, Zoubeidi A. A novel anti-androgen, Compound 30, suppresses castration-resistant and MDV3100-resistant prostate cancer growth in vitro and in vivo. *Mol Cancer Ther*. 2013 May;12(5):567-76.

3. Thomas C, Lamoureux F, Crafter C, Davies BR, Beraldi E, Fazli L, Kim S, Thaper D, Gleave ME, Zoubeidi A. Synergistic targeting of PI3K/AKT-pathway and androgen-receptor axis significantly delays castration-resistant prostate cancer progression in vivo. *Mol Cancer Ther*. 2013 Aug 21.

4. Zoubeidi A, et al. Cooperative interactions between androgen receptor (AR) and heat-shock protein 27 facilitate AR transcriptional activity. *Cancer Res*: 67(21): p. 10455-65 (2007).

5. Zoubeidi A, et al. The Fer tyrosine kinase cooperates with interleukin-6 to activate signal transducer and activator of transcription 3 and promote human prostate cancer cell growth. *Mol Cancer Re:* 7(1): p. 142-55 (2009).

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

Terry Fox New Frontiers Program Project (2012-2017)

Project Leader: John Bell, OHRI

Investigators: Harold Atkins, Jean-Simon Diallo, OHRI; David Stojdl, Children's Hospital of Eastern Ontario; Brad Nelson, Deeley Research Institute/BC Cancer Agency; Patrick Lee, Dalhousie University; Nahum Sonenberg, McGill University; Brian Lichty, Dr. Jonathan Bramson, Dr. Yonghong Wan, Dr. Karen Mossman, McMaster University; Andrea McCart, UHN

Scientific Summary: Our program is directed toward the discovery and testing of novel replicating anti-cancer viruses, and complimentary biotherapeutic strategies for the treatment of cancer. We are focused on the most promising oncolytic therapeutics including three that are currently in clinical development. We are also exploring cutting-edge immunotherapies including genetically modified immune cell platforms and personalized anti-tumour vaccines. We are carrying out fundamental studies to understand how viral therapeutics interact with the host, the tumour and tumour microenvironment. Small-molecule screening and functional genomics approaches are being used to identify therapeutic targets that can be modified to enhance virus killing of tumour cells.
Our ongoing studies include:

- Understanding how best to manipulate the host immune system to both facilitate virus delivery and potentiate anti-tumour immune responses.
- Delineating signaling pathways that sensitize tumour vascular endothelium and cancer-associated fibroblasts to virus infection and destruction.
- Characterization of the innate anti-viral response in normal and tumour cells that determine virus selectivity.
- Identification of tumour-specific pathways that can sensitize cancer cells to virus killing.
- Mechanism of action of small molecules that uniquely inactive the anti-viral interferon response.
- Role of protein translation regulation in determining virus therapeutic activity.

List of Key Publications:

1. Ottolino-Perry K, Tang N, Head R, Ng C, Arulanandam R, Angarita FA, Acuna SA, Chen Y, Bell J, Dacosta RS, McCart JA. Tumour vascularization is critical for oncolytic vaccinia virus treatment of peritoneal carcinomatosis. *Int J Cancer*. 2013 Jul 24 [epub ahead of print].

2. Conrad DP, Tsang J, Maclean M, Diallo JS, Le Boeuf F, Lemay CG, Falls TJ, Parato KA, Bell JC, Atkins HL. Leukemia cell-rhabdovirus vaccine: personalized immunotherapy for acute lymphoblastic leukemia. *Clin Cancer Res*. 2013 Jul 15; 19(14):3832-43.

3. Heo J, Reid T, Ruo L, Breitbach CJ, Rose S, Bloomston M, Cho M, Lim HY, Chung HC, Kim CW, Burke J, Lencioni R, Hickman T, Moon A, Lee YS, Kim MK, Daneshmand M, Dubois K, Longpre L, Ngo M, Rooney C, Bell JC, Rhee BG, Patt R, Hwang TH, Kirn DH. Randomized dose-finding clinical trial of oncolytic immunotherapeutic vaccinia JX-594 in liver cancer. *Nat Med*. 2013 Mar; 19(3):329-36.

4. Breitbach CJ, Arulanandam R, De Silva N, Thorne SH, Patt R, Daneshmand M, Moon A, Ilkow C, Burke J, Hwang TH, Heo J, Cho M, Chen H, Angarita FA, Addison C, McCart JA, Bell JC, Kirn DH. Oncolytic vaccinia virus disrupts Tumour-associated vasculature in humans. *Cancer Res*. 2013 Feb 15; 73(4):1265-75.

5. Bridle BW, Chen L, Lemay CG, Diallo JS, Pol J, Nguyen A, Capretta A, He R, Bramson JL, Bell JC, Lichty BD, Wan Y. HDAC inhibition suppresses primary immune responses, enhances secondary immune responses, and abrogates autoimmunity during Tumour immunotherapy. *Mol Ther*. 2013 Apr; 21(4):887-94.

6. Russell JC, Peng KW, Bell JC. Oncolytic virotherapy. *Nat Biotechnol*. 2012 Jul 10; 30(7):658-70.

7. CG Lemay, Rintoul JL, Kus A, Paterson JM, Garcia V, Falls TJ, Ferreira L, Bridle BW, Conrad DP, Tang VA, Diallo JS, Arulanandam R, Le Boeuf F, Garson K, Vanderhyden BC, Stojdl DF, Lichty BD, Atkins HL, Parato KA, Bell JC, Auer RC. Harnessing oncolytic virus-mediated antiTumour immunity in an infected cell vaccine. *Mol Ther*. 2012 Sep; 20(9):1791-9.

8. Yebdri FB, Vangrevenynghe J, Tang VA, Goulet ML, Wu J, Stojdl DF, Hiscott J, Lin R. Triptolide mediated-inhibition of interferon signaling enhances vesicular stomatitis virus based oncolysis. *Mol Ther*. 2013 Aug 28 [epub ahead of print].
9. Le Boeuf F, Batenchuk C, Vähä-Koskela M, Breton S, Roy D, Lemay C, Cox J, Abdelbary H, Falls T, Waghray G, Atkins H, Stojdl D, Diallo JS, Kærn M, Bell JC. Model-based rational design of an oncolytic virus with improved therapeutic potential. *Nat Commun*. 2013; 4:1974.

10. McGray AJ, Bernard D, Hallett R, Kelly R, Jha M, Gregory C, Bassett JD, Hassell JA, Pare G, Wan Y, Bramson JL. Combined vaccination and immunostimulatory antibodies provides durable cure of murine melanoma and induces transcriptional changes associated with positive outcome in human melanoma patients. *Oncoimmunology*. 2012 Jul 1; 1(4):419-31.

11. Pan D, Marcato P, Ahn DG, Gujar S, Pan LZ, Shmulevitz M, Lee PW. Activation of p53 by chemotherapeutic agents enhances reovirus oncolysis. *PLoS One*. 2013; 8(1):e54006.

12. Gujar S, Dielschneider R, Clements D, Helson E, Shmulevitz M, Marcato P, Pan D, Pan LZ, Ahn DG, Alawadhi A, Lee PW. Multifaceted therapeutic targeting of ovarian peritoneal carcinomatosis through virus-induced immunomodulation. *Mol Ther*. 2013 Feb; 21(2):338-47.

13. Borrego-Diaz E, Mathew R, Hawkinson D, Esfandyari T, Liu Z, Lee PW, Farassati F. Pro-oncogenic cell signaling machinery as a target for oncolytic viruses. *Curr Pharm Biotechnol.* 2012 Jul; 13(9):1742-9.

14. Sonenberg N, Hay N. Cancer genomics: the post-transcriptional era. *Curr Opin Genet Dev*. 2013 Feb; 23(1):1-2.

15. Alain T, Morita M, Fonseca BD, Yanagiya A, Siddiqui N, Bhat M, Zammit D, Marcus V, Metrakos P, Voyer LA, Gandin V, Liu Y, Topisirovic I, Sonenberg N. eIF4E/4E-BP ratio predicts the efficacy of mTOR targeted therapies. *Cancer Res*. 2012 Dec 15; 72(24):6468-76.

16. West NR, Kost SE, Martin SD, Milne K, Deleeuw RJ, Nelson BH, Watson PH. Tumour-infiltrating FOXP3(+) lymphocytes are associated with cytotoxic immune responses and good clinical outcome in oestrogen receptor-negative breast cancer. *Br J Cancer*. 2013 Jan 15; 108(1):155-62.

The Terry Fox New Frontiers Program Project Grant in Ultrasound for Cancer Therapy

Terry Fox New Frontiers Program Project Grant (2010-2013)

Investigators: Gregory J. Czarnota, Jean Philippe Pignol, Eileen Rakovitch, Gregory J. Stanisz, Sunnybrook Research Institute; Kristy Brock, UHN, UofT; Michael C. Kolios and Raffi Karshafian, Ryerson University.

Scientific Summary: We are proposing to develop and enhance the use of ultrasound techniques to improve cancer treatment. We will continue developing quantitative ultrasound methods to detect and track the progression of the effects of cancer therapies on cell death. In addition, we will develop novel ultrasound-based therapies in order to improve current conventional cancer treatments. The ultimate goal is to apply both aims integrated clinically at the end of the proposed research period, with clinical evaluations of enhanced imaging and cancer treatment derived from these research objectives.

There are four interrelated complementary projects proposed that are critical to bringing these technologies to the clinic. The first will see the continued development of quantitative ultrasound methods for the detection of tumour responses to cancer therapies at high and low frequency, for preclinical and clinical applications, respectively. The second will focus on correlative analyses with quantitative ultrasound approaches and will focus on correlating dynamic contrast enhanced MRI tumour data, and whole mount three-dimensional histopathological data, with the ultrasound data. The third project will be centered about evaluating quantitative ultrasound data from patients receiving cancer therapy using established methods in quantitative ultrasound and be guided by ongoing developments from the first two projects. The last project will further develop recent innovations in using ultrasound as an enhancing agent for cancer therapy based on our discovery of bubble-enhanced ultrasound potentiation of tumour response.

The overarching goal is to use our ultrasound methods to detect tumour responses to therapy. If a therapy is detected early on to be ineffective such methods could be used to guide changes from ineffective therapies to more efficacious ones. Such therapies can include chemotherapy, radiation therapy or new therapies including those to be developed as part of this application.

List of Key Publications:

1. Briggs K, Al Mahrouki A, Nofiele J, El-Falou A, Stanisz M, Kim HC, Kolios MC, Czarnota GJ, "Non-invasive Monitoring of Ultrasound-Simulated Microbubble Radiation Enhancement Using Photoacoustic Imaging," Technology in cancer research and treatment; PubMed ID:24000993; Aug. 2013.

2. Kwok SJ, El Kaffas A, Lai P, Al Mahrouk A, Lee J, Iradji S, Tran WT, Giles A, Czarnota GJ, "Ultrasound-Mediated Microbubble Enhancement of Radiation Therapy Studied Using Three-Dimensional High-Frequency Power Doppler Ultrasound," Ultrasound in medicine and biology; PubMedID: 23993051; Aug. 2013.

3. Kim HC, Al Mahrouki A, Gorjizadeh A, Karshafian R, Czarnota GJ, "Effects of biophysical parameters in enhancing radiation responses of prostate tumours with ultrasound-stimulated microbubbles," Ultrasound in medicine and biology, 39(8); PubMedID: 23643061; Aug. 2013.

4. Sadeghi-Naini A, Papanicolau N, Falou O, Tadayyon H, Lee J, Zubovits J, Sadeghian A, Karshafian R, Al Mahrouki A, Tran W, Papanicolau N, Kolios MC, Czarnota GJ, "Conventional frequency ultrasound biomarkers of cancer treatment response in vivo," Translational oncology, 6(3); PubMedID:23761215; June 2013.

5. Kolios Mc, Berndl ES, Wirtzeld LC, Strohn EM, Czarnota GJ, " Acoustic and photoacoustic imaging of spheroids," The Journal of the Acoustical Society of America, 133(5); PubMedID:23655729; May 2013.

6. Sadeghi-Naini A, Falou O, Czarnota GJ, "Characterizing tumour heterogeneous response to chemotherapy using low-frequency ultrasonic spectroscopy," The Journal of the Acoustical Society of America 133 (5); PubMedID: 23655931; May 2013.

7. Sadeghi-Naini A, Papanicolau N, Falou O, Zubovits J, Dent R, Verma S, Trudeau M, Boileau JF, Spayne J, Iradji S, Sofroni E, Lee J, Lemon-Wong S, Yaffe M, Kolios MC, Czarnota GJ, "Quantitative ultrasound evaluation of tumour cell death response in locally advanced breast cancer patients receiving chemotherapy," Clinical cancer research: An Official Journal of the American Association for Cancer Research, 19 (8); PubMedID: 23426278; April 2013.

8. El Kaffas A, Giles A, Czarnota GJ, "Dose-dependent response of tumour vaculature to radiation therapy in combination with Sunitinib depicted by three-dimensional high-frequency power Doppler ultrasound," Angiogenesis, 16(2); PubMedID: 23314761, April 2013.

9. Falou O, Sadeghi-Naini A, Prematilake S, Sofroni E, Papanicolau N, Iradji S, Jahedmotlagh Z, Lemon-Wong S, Pignol JF, Wright FC, Yaffe MJ, Czarnota GJ, "Evaluation of neoadjuvant chemotherapy response in women with locally advanced breast cancer using ultrasound elastography," Translational Oncology, 6(1); PubMedID: 23418613.

10. Nofiele JT, Karshafian R, Furukawa M, Al Mahrouki A, Giles A, Wong S, Czarnota GJ, "Ultrasound-activitated microbubble cancer therapy: ceramide production leading to enhanced radiation effect in vitro," Technology in Cancer Research and Treatment, 12(1); PubMedID: 22905807; Feb. 2013

11. Al Mahrouki A, Karshafian R, Giles A, Czarnota GJ, "Bioeffects of ultrasound-stimulated microbubbles on endothelial cells: gene expression changes associated with radiation enhancement in vitro," Ultrasound in medicine and biology, 38(11); PubMedID: 22980406; Nov. 2012.

12. El Kaffas A, Tran W, Czarnota GJ, "Vascular strategies for enhancing tumour response to radiation therapy," Technology in cancer research and treatment, 11(5); PubMedID: 22568629; Oct. 2012.

13. Falou O, Soliman H, Sadeghi-Naini A, Iradji S, Lemon-Wong S, Zubovits J, Spayne J, Dent R, Trudeau M, Boileau JF, Wright FC, Yaffe MJ, Czarnota, GJ, " Diffuse optical spectroscopy evaluation of treatment response in women with locally advanced breast cancer receiving neoadjuvant chemotherapy," Translational oncology, 5(4); PubMedID: 22937175;Aug. 2012.

14. Czarnota GJ, Karshafian R, Burns PN, Wong S, Al Mahrouki A, Lee JW, Caissie A, Tran W, Kim C, Furukawa M, Wong E, Giles A, "Tumour radiation response enhancement by acoustical stimulation of the vascalature," Proceedings of the National Academy of Sciences of the United States of America, 109(30); PubMedID: 22778441; July 2012.

15. Lee J, Karshafian R, Papanicolau N, Giles A, Kolios MC, Czarnota GJ, "Quantitative ultrasound for the monitoring of novel microbubble and ultrasound radiosensitization," Ultrasound in medicine and biology 38(7); PubMedID: 22579547; July 2012.

16. Falou O, Sadeghi-Naini A, Soliman H, Yaffe MJ, Czarnota GJ, "Diffuse optical imaging for monitoring treatment response in breast cancer patients," Conference Proceedings:...Annual International Conference of the IEEE Engineering in Medicine and Biology Society. IEEE Engineering in Medicine and Biology Society, Conference, 2012; PubMedID: 23366595; Jan. 2012.

17. Sadeghi-Naini A, Falou O, Czarnota, GJ, "Quantitative ultrasound spectral parametic maps: early surrogates of cancer treatment response," Conference Proceedings:...Annual International Conference of the IEEE Engingeering in Medicine and Biology Society. IEEE Engineering in Medicine and Biology Society, Conference, 2012; PubMedID: 23366475; Jan. 2012.

18. Bailey C, Desmond KL, Czarnota GJ, Stanisz GJ. Quantitative magnetization transfer studies of apoptotic cell death. *Magn Reson Med:* Jul; 66(1):264-9, 2011. doi: 10.1002/mrm.22820. Epub 2011 Feb 17.

19. Vlad RM, Kolios MC, Czarnota GJ. Ultrasound imaging of apoptosis: spectroscopic detection of DNA-damage effects at high and low frequencies. *Methods Mol Biol:* 682:165-87 (2011).

20. Strohm E, Czarnota GJ, Kolios MC. Quantitative measurements of apoptotic cell properties using acoustic microscopy. *IEEE Trans Ultrason Ferroelectr Freq Control.* Oct; 57(10):2293-304 (2010).

21. Vlad RM, Kolios MC, Moseley JL, Czarnota GJ, Brock KK. Evaluating the extent of cell death in 3D high frequency ultrasound by registration with whole-mount tumour histopathology. *Med Phys:* Aug; 37(8):4288-97 (2010).

22. Vlad RM, Saha RK, Alajez NM, Ranieri S, Czarnota GJ, Kolios MC. An increase in cellular size variance contributes to the increase in ultrasound backscatter during cell death. *Ultrasound Med Biol*: Sep; 36(9):1546-58 (2010).

The Terry Fox New Frontiers Program Project in Addressing Tumour Heterogeneity Through Identification of Subgroup-Specific "Shared Maintenance Genes": The Right Target for Each Cancer *(2011-2014)*

Investigators: Sean Egan, Michael Taylor, Hospital for Sick Children, UofT; James Woodgett, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, UofT; Eldad Zacksenhaus, Toronto General Research Institute, UofT

Scientific Summary: The majority of cancer deaths occur secondary to metastatic disease. Despite this, cancer therapy so far has been based on analyses of primary tumours. The assumption has been that biological insights gained from studying a primary tumour will apply to its metastatic descendents. However, there is strong evidence that metastases can disseminate early and develop independently in parallel to primary disease. Indeed, a recent study has shown that breast cancer metastases can diverge considerably from primary tumour. In addition, the Taylor lab has found that human and mouse metastatic medulloblastoma share only a minority of genetic events in common with their matching primary tumour. *These data strongly suggest that targeted therapies based on studying the primary tumour in isolation and without consideration of metastases are unlikely to be curative.*

We hypothesize that the best target(s) for rationale therapy to treat both primary and metastatic disease will lie within the sub-set of mutational events that is shared by a primary tumour and its metastases, and which is critical for tumour maintenance of both.

We will define these critical Shared Maintenance Genes for cancers of the brain and breast through the following projects and Cores:

- Identification of Shared Maintenance Genes in Shh Medulloblastoma
- Identification of Shared Maintenance Genes in p53 and IKKε-Induced Breast Cancer
- Assessment of Wnt and PI3K Signaling Components as Shared Maintenance Genes in Primary and Metastatic Medulloblastoma and Breast Cancer
- Identification of Shared Maintenance Genes in Triple Negative Breast Cancer
- Xenograph and Histology Core
- Genomics and Bioinformatics Core

List of Key Publications:

1. Liu J., Egan S.E., and Zacksenhaus E. A tumor initiating cell-derived prognostic signature for HER2+:ERα- breast cancer; rationale, new features, controversies and future directions. Oncotarget. 2013 Jul 26. [Epub ahead of print] PMID: 23945331

2. Tyler J.W. Robinson, Melody Pai, Jeff C. Liu, Frederick Vizeacoumar, Thomas Sun, Sean E. Egan, Alessandro Datti, Jing Huang, and Eldad Zacksenhaus. High-content screen identifies disulfiram as a potential therapeutic that cooperates with doxorubicin to target triple-negative breast cancer stem cells: interaction with IQ-motif-containing factors. Cell Cycle, 2013, 2013 Aug 12;12(18). PMID: 23974104

3. Tyler J. W. Robinson, Jeff C. Liu, Frederick Vizeacoumar, Thomas Sun, Sean E. Egan, Aaron Schimmer, Alessandro Datti and Eldad Zacksenhaus. RB1 status in triple negative breast cancer dictates response to radiation treatment and selective therapeutic drugs. Plos one, accepted with minor revisions.

4. Northcott PA. et al. Subgroup-Specific Structural Variation Across 1000 Medulloblastoma Genomes. Nature. 2012 Aug 2;488(7409):49-56. doi: 10.1038/nature11327 (MDT is senior corresponding author). PMID: 22832581

5. Leprivier G. et al. The eEF2 kinase confers resistance to nutrient deprivation by blocking translational elongation. Cell 2013. 153(1064-1079). PMID: 23706743

6. Northcott PA, Jones DTW, Kool M, Robinson GW, Gilbertson RJ, Cho YJ, Pomeroy SL, Korshunov A, Lichter P, Taylor MD, Pfister SM. Medulloblastomics, the end of the beginning. Nature Reviews Cancer 213 (818-34). PMID 23175129 (MDT is co-corresponding author).

The Terry Fox New Frontiers Program Project Grant in Molecular Correlates of Treatment Failure in Lymphoid Cancers *(2013-2016)*

Project Leader: Randy Gascoyne, BC Cancer Agency

Investigators: Marco Marra, BC Cancer Agency, Genome Science Centre; Sohrab Shah, BC Cancer Agency; Christian Steidl, Joseph Connors, BC Cancer Agency

Scientific Summary: Most lymphoid cancers are easily treated and, in specific subtypes, treatment is given with curative intent. Diffuse large B-cell lymphoma (DLBCL) accounts for 30 to 40% of all non-Hodgkin lymphomas (NHL) and is curable even when widely disseminated at the time of diagnosis. Seminal work from our group has recently characterized the mutational landscape of these tumours and now the current renewal of this TFRI New Frontiers Program Project Grant will investigate at unprecedented resolution, the molecular correlates of treatment failure through the study of clinical samples from patients who were not cured with state-of-the-art therapy. Similar studies will also be conducted in follicular lymphoma (FL), the second most common form of NHL.

The BC Cancer Agency is recognized as a world leader in using next-generation sequencing technologies of lymphoid cancers to understand the fundamental biology and identify the recurrent genetic abnormalities (so-called driver mutations) that represent the underpinnings of these cancers. Building on previous work from our group, we will leverage prior discoveries to determine if tumours from patients with primary treatment failure are different from those experiencing complete remissions through the study of relapsed or recurrent disease. We hypothesize that a limited number of cellular pathway perturbations underlie the biology of treatment failure and by studying these cases we will identify the candidate molecules and pathways that could be used to explore novel, targeted therapies. We plan to study the functional consequences of these genetic alterations and develop a small suite of tests that could be used to recognize at the time of diagnosis those patients destined to not be cured with current treatments. We strongly believe that these studies will ultimately improve the outcome for patients with both DLBCL and FL and fulfill our mandate of delivering precision medicine for patients with lymphoid cancers.

List of Key Publications:

1. Morin RD, Mungall K, Pleasance E, Mungall AJ, Goya R, Huff R, Scot DW, Ding J, Roth A, Chiu R, Corbett RD, Chan FC, Mendez-Lago M, Trinh DL, Bolger-Munro M, Taylor G, Hadj Khodabakhshi A, Ben-Neriah S, Pon J, Meissner B, Woolcock B, Farnoud N, Rogic S, Lim E, Johnson NA, Shah S, Jones S, Steidl C, Holt R, Birol I, Moore R, Connors JM, Gascoyne RD, Marra MA. Mutational and structural analysis of diffuse large B cell lymphoma using whole genome sequencing. Blood (epub May 22, 2013) (PMID: 23699601).

2. Trinh DL, Scott DW, Morin RD, Mendez-Lago M, An J, Jones SJM, Mungall AJ, Zhao Y, Schein J, Steidl C, Connors JM, Gascoyne RD, Marra MA. Analysis of FOXO1 mutations in diffuse large B cell lymphoma. Blood 121(18): 3666-74 (2013) (PMID: 23460611).

3. Johnson NA, Slack GW, Savage KJ, Connors JM, Ben-Neriah S, Rogic S, Scott DW, Tan KL, Steidl C, Hosrman DE, Sehn LH, Chan WC, Iqbal J, Meyer P, Lenz G, Wright G, Rimsza LM, Valentino C, Brunhoeber P, Grogan TM, Braziel RM, Cook JR, Tubbs RR, Weisenburger DD, Campo E, Rosenwald A, Ott G, Delabie J, Jaffe ES, Staudt LM, Gascoyne RD. Concurrent expression of MYC and BCL2 in R-CHOP treated diffuse large B cell lymphoma. J Clin Oncol 28: 3452-3459 (2012) (PMID: 22851565).

The Terry Fox New Frontiers Program Project Grant in Prostate Cancer Progression

(2011-2016)

Investigators: Martin Gleave, Colin Collins, Emma Guns, Michael Cox, Chris Ong, Paul Rennie, Amina Zoubeidi, Yuzhuo Wang, VPC,UBC; Kim Chi, Shoukat Dedhar, Poul Sorensen, BC Cancer Agency

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 20 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.
- Since our ongoing TF program cycle renewal in December 2011, we have published over 150 papers, applied for or obtained >50 patents, and are in the process of out licensing a novel therapeutic to a new spin-off company. We have enrolled over 250 patients in clinical trials, and completed 10 Phase I and II trials of novel agents discovered as a direct consequence of pre-clinical laboratory research performed under the auspices of the Program.

Leading in this regard is OGX-011, now in global Phase III trials after demonstration of a seven-month survival gain in the randomized Phase II NCIC IND.165 trial. Patient accrual has been completed in the SYNERGY trial using OGX-011 as first-line therapy in metastatic Castrate-Resistant Prostate Cancer (CRPC), while two other Phase III studies (AFFINITY (second line CRPC) and ENSPIRIT (NSCLC)) are currently enrolling patients. We also led the bench-bedside translation of a second novel inhibitor targeting Hsp27, OGX-427, which is currently in six randomized Phase II studies in CRPC, bladder, NSCLC and pancreatic cancer. Patient enrolment in the Borealis-1 TM Trial of OGX-427 in Metastatic Bladder Cancer has recently been completed. This direct translation of basic science to the clinic is how our team works and is in keeping with the mandate of the Terry Fox Foundation.

Our program consists of six individual but highly integrated research projects grouped into four relational areas:

- Target Discovery: Project #1 is using next-generation sequencing technologies to identify changes in the genomes and transcriptomes of tumours mechanistically linked to castrate resistance, with a particular focus on androgen receptors and androgen biosynthetic pathways.
- Cell Biology Mechanisms: Project #2 is continuing studies on the stress response and cytoprotective chaperones in treatment resistance, focusing on the role of clusterin in endoplasmic reticular stress and autophagy. Project #3 is investigating mechanisms of ERG-mediated prostatic carcinogenesis and progression.
- Molecular & Cellular Targets: Projects #4 and #5 are investigating the anti-apoptosis proteins, BIRC6, and Semaphorin 3C, respectively, in treatment resistance of prostate cancer and as potential therapeutic targets.
- Clinical Evaluation: Project #6 is performing correlative measures to test biologic activity for a combination of a potent anti-androgen in combination with an inhibitor of clusterin (OGX-011) or Hsp27 (OGX-427, both developed in our Program) in a Phase II clinical trial in CRPC patients. In this regard we have initiated a multiCenter randomized trial of OGX-427 +/- abiraterone in post ABI CRPC. All sub-projects within the Terry Fox program are supported by a shared core facility with five major components: Advanced Genomics & Bioinformatics, Pathology & Molecular Imaging, Animal Models, Analytical Pharmacology, and Translational Trials.

In summary, our Program Project Grant on Prostate Cancer Progression is a major catalyst for translational research that has enabled us to have already brought several new therapies from bench to bedside.

The program ongoing for the next four years 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:

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The Terry Fox New Frontiers Program Project Grant in Core Pathogenic Pathways in Human Leukemia *(2012-2017)*

Investigators: R. Keith Humphries, Connie J. Eaves, Aly Karsan and Andrew Weng, BC Cancer Agency; Martin Hirst, UBC

Scientific Summary: Our ultimate goal is to resolve, at high resolution, molecular differences between normal and leukemic hematopoietic stem cells that explain the abnormal properties of the latter and will allow novel therapeutic targets to be identified. To this end we are developing novel methods to reproducibly create genetically defined models of aggressive leukemias directly from normal *human* hematopoietic stem cells in the laboratory. Our group now brings powerful genetic engineering tools to enable human models of aggressive leukemia to be rapidly created in the lab so that mechanisms of treatment resistance and new drugs and biomarkers can be efficiently analyzed and tested directly and repeatedly in human cells that mimic, but do not rely on, patients' cells. We have made significant progress in developing several novel methods for modeling the generation of acute myeloid and T-cell leukemia and progression to frank leukemia from chronic myelogenous leukemia or myelodysplastic syndrome. These new models now give us enormous power to explore the differences between normal and leukemic hematopoietic stem cells at multiple levels. The next step is to build on these models to pinpoint the critical changes that occur in cells leading to their transformation to leukemia and hence to better resolve potential new therapeutic targets. The program consists of three complementary and synergistic projects and two core facilities that span a spectrum of approaches to this overarching theme, including:

- Sub-project one: to exploit lenti-viral gene transfer and the potent transforming properties of HOX/MEIS1/MN1 and other oncogenes to first create and then exploit 2 novel models of human AML representative of de novo AML and progression of chronic phase CML to myeloid blast crisis.
- Sub-project two: to understand the genomic events that drive leukemogenesis arising in a clonal background of MDS.
- Sub-project three: characterize pathogenetic programs in human T-ALL that drive initial malignant transformation of normal progenitors, propagation of established clones, and resistance to standard chemotherapy.
- Cell, Vector and Mouse Core: serve as a provider of quality-controlled cells, reagents (including a vast array of lenti-viral vectors), specialized mouse strains and protocols that will be used by all projects and essential training.
- Epigenomics core: work cooperatively with other program investigators to identify shared epigenetic mechanisms and pathways used by primitive human LSCs through the provision and continuing development of cutting edge epigenomic and transcriptome profiling technologies.

List of Key Publications:

1. Giambra V, Jenkins CR, Wang H, Lam SH, Shevchuk OO, Nemirovsky O, Wai C, Gusscott S, Chiang MY, Aster JC, Humphries RK, Eaves C & Weng AP. NOTCH1 promotes T cell leukemia-initiating activity by RUNX-mediated regulation of PKC-q and reactive oxygen species. *Nat Med* 18: 1693-8, 2012

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6. Miller PH, Knapp DJ & Eaves CJ. Heterogeneity in hematopoietic stem cell population: implications for transplantation. *Curr Opin Hematol* 23:859-862, 2013. (PMID: 23615054)

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The Terry Fox New Frontiers Program Project Grant in the Genomics of Forme Fruste Tumours: New Vistas on Cancer Biology and Management *(2013-2018)*

Investigators: David Huntsman, Samuel Aparicio, Sohrab Shah, Poul Sorensen, BC Cancer Agency,UBC; Carl Hansen, Michael Underhill, UBC; Martin Hirst, Marco Marra, Gregg Morin, Michael Smith Genome Sciences Center (MSGSC) and UBC; Chenghan Lee, University of Alberta; Ryan Morin, MSGSC and SFU; Torsten Nielsen, Vancouver General Hospital (VGH),UBC; Stephen Yip, Anna Tinker, BC Cancer Agency, UBC; Paul Clarkson, BC Cancer Agency; Jessica McAlpine, David Schaefer, VGH

Scientific Summary: The New Frontiers Program Project Grant in the Genomics of Forme Fruste Tumours represents a new frontier for attacking the cancer problem through the unique perspective gained from the study of forme fruste tumour types and using state-of-the-art technology to achieve our objectives. Forme fruste tumours, which are the focus of this research program, are clinically and pathologically homogenous tumour types, presumed to be 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, information derived from the study of these cancers often has broader clinical relevance. Our team, which was initially funded in 2010, has an exceptional track record of identifying mutations that are characteristic of forme fruste tumours and using this knowledge both to improve management of these tumours and also to provide broader insights into cancer biology. We have developed four inter-related sub-projects and one core facility that will work together to study these cancers.

Sub-project one is the main sequencing/discovery project and will generate a complete catalogue of genomic alterations in forme fruste tumours using a comprehensive suite of next generation sequencing techniques.
Sub-project two will combine some of the deep sequencing data from Sub-project one with newly generated sequencing data for selected forme fruste tumours with known alterations to comprehensively describe the clonal sub-populations within these tumour types. This deep sequencing data will be analysed to characterize the genotype of individual cell populations within the tumours and how the different clonal mutation profiles have evolved. As part of this sub-project, model xenograft systems will be studied, pre- and post- treatment, to determine how these tumours evolve under the pressure of targeted therapeutics.
Sub-project three will use isogenic tumour cell line models to validate how genomic alterations identified in Sub-projects one and two will affect the epigenome, mutant protein expression, protein interaction networks, and tumourigenic cellular phenotypes. Sequence based methods will query the epigenome, and proteomic methods will identify effects on protein networks and the translatome. Drug screens will be used to confirm therapeutically actionable targets and processes.
Sub-project four will optimize methods for measuring levels of circulating tumour DNA in forme fruste cancers as a novel diagnostic and tumour monitoring tool. The circulating tumour DNA levels will be correlated with clinical parameters. These four sub-projects will be supported by a Data Analysis Core for bioinformatics analysis, statistical analysis, and data analysis. The discoveries from this project will be translated into the clinic through our collaboration with the SMART (Shared Access Medicine: An Approach to Rare Tumours), through the Center for Drug Research and Development, and through collaborations with clinical trials groups. 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. Anglesio, M., Y. Wang, W. Yang, J. Senz, A. Wan, A. Heravi-Moussavi, C. Salamanca, S. Maines-Bandiera, D. Huntsman, and G. Morin, Cancer-associated somatic DICER1 hotspot mutations cause defective miRNA processing and reverse strand expression bias to predominantly mature 3p strands through loss of 5p strand cleavage. *J Pathol*, 2012.

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Maines-Bandiera, C. Salamanca, B. Pasini, B.A. Clarke, A.F. Lee, C.H. Lee, C. Zhao, R.H. Young, S.A. Aparicio, P.H. Sorensen, M.M. Woo, N. Boyd, S.J. Jones, M. Hirst, M.A. Marra, B. Gilks, S.P. Shah, W.D. Foulkes, G.B. Morin, and D.G. Huntsman, Recurrent somatic DICER1 mutations in nonepithelial ovarian cancers. *N Engl J Med*, 2012. 366(3): p. 234-42.

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14. Wiegand, K.C., S.P. Shah, O.M. Al-Agha, Y. Zhao, K. Tse, T. Zeng, J. Senz, M.K. McConechy, M.S. Anglesio, S.E. Kalloger, W. Yang, A. Heravi-Moussavi, R. Giuliany, C. Chow, J. Fee, A. Zayed, L. Prentice, N. Melnyk, G. Turashvili, A.D. Delaney, J. Madore, S. Yip, A.W. McPherson, G. Ha, L. Bell, S. Fereday, A. Tam, L. Galletta, P.N. Tonin, D. Provencher, D. Miller, S.J. Jones, R.A. Moore, G.B. Morin, A. Oloumi, N. Boyd, S.A. Aparicio, M. Shih Ie, A.M. Mes-Masson, D.D. Bowtell, M. Hirst, B. Gilks, M.A. Marra, and D.G. Huntsman, ARID1A mutations in endometriosis-associated ovarian carcinomas. *N Engl J Med*, 2010. 363(16): p. 1532-43.

The Terry Fox New Frontiers Program Project Grant in Defining and Applying Oncometabolism: A Team Approach in Understanding and Translating the Warburg Effect from Oncogenic and Tumour Suppressing Activities *(2011-2014)*

Investigators:

1. R. Jones, N. Beauchemin (McGill University): Investigating how the AMP-activated protein kinase (AMPK) influences the Warburg effect in cancer.
2. A. Pause, R. Jones (McGill University): How BHD/Folliculin interact with AMPK in a novel nutrient sensing pathway.
3. N. Sonenberg, M. Pollak, I. Topisirovic (McGill University): Metabolic targeting of cancer using *metformin/phenformin* in combination with *rapalogues*.
4. M.L. Tremblay, S. Hardy (McGill University): Investigating protein tyrosine phosphatases (PTPs) that modulate AMPK-related metabolic/signaling pathways.
5. J. St-Pierre, V. Giguère (McGill University): Defining the transcriptional network controlled by the ERRa/PGC-1b pathway that favours the Warburg effect.
6. D. Avizonis, O. Mamer, R. Nadon (McGill University): The Metabolomic Core Facility (metabolome profiling, bioinformatics, and assay development).

Scientific Summary: Since Otto Warburg's observation 80 years ago that cancer cells use sugar in a different manner from normal cells, and in fact use it to fuel their own growth, extensive research has focused on understanding the energy metabolism of cancer cells. It is clear that rapidly dividing cells, including cancer cells, require a plethora of metabolites that extend far beyond the need of ATP to generate proteins, nucleic acids and lipids. Understanding the metabolic differences in cancer cells, or oncometabolism, is one of the emerging areas of research for the development of potential new cancer therapeutics. Research focused on identifying the unique metabolic profiles of tumour cells may allow for a more rational design of single or combinatorial targeted therapies. All five research projects that make up this Terry Fox Foundation oncometabolism project focus on various aspects of cell growth and proliferation networks and their impact on cancer metabolism. Three of the five projects (Jones and Beauchemin; Pause and Jones; Sonenberg, Pollak, and Topisirovic) have directly addressed the importance of the metabolic regulator AMPK for both tumour development and tumour metabolism. The fourth project (Tremblay and Hardy) is designed to identify PTPase family members that modulate biosynthetic and signalling pathways. Finally, the fifth research program (St.-Pierre and Giguère) uses functional genomics to examine the transcriptional network controlled by the ERRa/PGC-1b pathway and its impact on tumour metabolism and proliferation. At the center of this scientific team is the Metabolomic Core Facility (MCF) led by Drs. Avizonis, Mamer, and Nadon. The MCF is a one-of-a-kind facility in Canada, as it is dedicated solely to investigating cancer metabolism. The facility offers a unique series of platforms that allows the complete characterization of cellular metabolism in both normal and cancerous cells. This group, by a team of basic and clinical researchers, is dedicated to the study of cancer bioenergetics and the identification of novel metabolic networks in cancer. Understanding the specific pathways that regulate cancer cell metabolism will lead to enhanced knowledge of cancer initiation and progression, and may help identify novel pathways and/or signatures of metabolites useful for developing novel diagnostics or therapeutic targets for cancer.

Scientific Progress: The oncometabolism team was awarded a new, three-year TFF-PPG in July 2011 and has made significant progress in just two years. One of the main achievements was the establishment of the metabolomics core to support the research of program members, which was aided by the recruitment of two highly skilled technologists (Luc Choinière and Gäelle Bridon). In addition to meeting the research needs of Oncometabolism team members, the core has developed new metabolomics methodologies (Mamer *et al.*, *Metabolomics*, *In Press*) and established over 50 national and international collaborations using technologies developed by the group. Within the context of the oncometabolism project, Drs. Jones and Beauchemin established that the AMPK energy sensing pathway mediates tumour suppression and metabolic reprogramming in cancer (Faubert *et al.*, *Cell Metab.*, 2013; Faubert *et al., PNAS,* In revision). They have also established that LKB1-AMPK signaling promotes the development of ErbB2-mediated breast cancer (Dupuy *et al.*, *Cancer & Metabolism*, 2013). Together, this work established that cellular energy sensors can influence tumourigenesis, and provides rationale for targeting energy-sensing pathways in cancer.

The team of Pause and Jones was focused on identifying novel pathways of stress resistance in cancer. They identified the tumour suppressor protein FLCN as an evolutionary conserved negative regulator of AMPK that promotes metabolic reprogramming in cancer (submitted to *PLoS Genetics* and *J. Clin. Invest.*, 2013). Together, these findings revealed a novel tumour suppressor mechanism for FLCN involving AMPK-dependent metabolic reprogramming. Over the past two years, Drs. Sonenberg, Pollak and Topisirovic demonstrated that biguanides exert their biological effects by altering translation of a specific subset of mRNAs including those that promote neoplastic proliferation (Larsson *et al.*, *PNAS*, 2012)**,** and that the anti-proliferative effects of biguanides are attenuated by the induction of glycolysis (Javeshghani *et al.*, *Cancer Res.*, 2012)**.** Finally, in collaboration with Dr. St.-Pierre, Drs. Sonenberg, Pollak and Topisirovic showed that the mTORC1/4E-BP pathway maintains cellular energy homeostasis by modulating translation of mRNAs encoding mitochondria-related genes (Morita *et al.*, *Cell Metab.*, In revision). Together these findings have provided additional mechanistic insights underlying the anti-neoplastic activity of biguanides. The group of Dr. Tremblay was focused on identifying protein tyrosine phosphatases (PTPs) that modulate metabolism and cellular biosynthesis in cancer cells. As part of the Oncometabolism PPG, Dr. Tremblay uncovered a specific role for the PRL subfamily of PTPs, the most oncogenic of all PTPs, in regulating AMPK activation and mitochondrial ATP production (manuscript in preparation). Finally, Drs. St.-Pierre and Giguère made significant progress understanding the influence of transcriptional networks on cellular metabolism in breast cancer. They discovered that the ERR/PGC axis regulates the expression of genes involved in glutamine metabolism ("glutaminolysis") to promote continued tumour growth under low oxygen conditions (submitted to *Cancer & Metabolism*, 2013). Another key discovery was the identification of mTOR as a central regulator of ERR activity (Chaveroux et al., *Cell Metab*., 2013). Drs. St.-Pierre and Giguère also wrote reviews (*Oncogene*, 2012; *Nature Rev. Cancer*, 2013) highlighting key recent discoveries and future research directions in cancer metabolism, many which form the basis of this grant application.

List of Key Publications:

1. McGuirk, S., Gravel, S.-P., Deblois, G., Faubert, B., Wegner, A., Hiller, K., Avizonis, D., Akavia, U.D., Jones, R.G., Giguère, V. and St-Pierre, J. (2013). PGC-1α supports glutamine-mediated lipogenesis in breast cancer. (Submitted).
2. Yan, M., Gingras, M.-C., Dunlop, E., Nouët, Y., Dupuy, F., Laëtitia, C., Jalali, Z., Possik, E., Faubert, B., Dydensborg, A.B., Sabourin, S., Kharitidi, D., Preston, R., Davies, D.M., Roughed, T., Jones, R.G., Tee, A., and Pause, A. (2013). Loss of folliculin tumour suppressor drives AMPK-dependent metabolic transformation. (Submitted).
3. Possik, E., Jalai, Z., Nouët, Y., Yan, M., Gingras, M.-C., Laëtitia, C., Dupuy, F., Panaite, L., Prezel, E., Kharitidi, D., Hall, D.H., Jones, R.G., and Pause, A. (2013). FLCN is a novel evolutionarily conserved negative regulator of AMPK-mediated stress responses. (Submitted).
4. Morita, M., Gravel, S.-P., Chénard, V., Sikström, K., Zheng, L., Alain, T., Avizonis, D., Zakaria, C., McLaughlan, S., Pollak, M., Gottlieb, E., Larsson, O., St-Pierre, J., Topisirovic, I. and Sonenberg, N. Translational control of the mitochondrion. *Cell Metab*. (in revision).
5. Faubert, B., Vincent, E.E., Griss, T., Svensson, R., Mamer, O.A., Avizonis, D., Shaw, R.J., and Jones, R.G. (2013). Loss of LKB1 promotes metabolic reprogramming of cancer cells via HIF1α. (In revision).
6. Mamer, O., Gravel, S.-P., Choinière, L., Chénard, V., St-Pierre, J. and Avizonis, D. (2013). The complete targeted profile of the organic acid intermediates of the citric acid cycle using a stable isotope dilution analysis, sodium borodeuteride reduction and selected ion monitoring GC/MS. *Metabolomics* (in press).
7. Chaveroux C, Eichner LJ, Dufour CR, Shatnawi A, Khoutorsky A, Bourque G, Sonenberg N, Giguère V. (2013). Molecular and genetic crosstalks between mTOR and ERRα are key determinants of rapamycin-induced nonalcoholic fatty liver. *Cell Metab*. Apr 2;17(4):586-98.
8. F. Dupuy, T. Griss, D. Avizonis, C. Ling, Z. Dong, D.R. Siwak, M.G. Annis, G.B. Mills, W.J. Muller, P.M. Siegel and R.G. Jones. (2013). LKB1 is a central regulator of tumour initiation and pro-growth metabolism in ErbB2-mediated breast cancer. *Cancer & Metabolism* 1:18.
9. Faubert, G. Boily, S. Izreig, T. Griss, B. Samborska, Z Dong, F. Dupuy,C. Chambers, B.J. Fuerth, B. Viollet, O. Mamer, D. Avizonis, R. DeBerardinis, P.M. Siegel, and R.G. Jones. (2013). AMPK is a Negative Regulator of the Warburg Effect and Suppresses Tumour Growth In Vivo. *Cell Metabolism*. 17(1):113-124.
10. Larsson, O., Morita, M., Topisirovic, I., Alain, T., Blouin, M. J., Pollak, M., and Sonenberg, N. (2012). Distinct perturbation of the translatome by the antidiabetic drug metformin. *Proc Natl Acad Sci U S A* 109, 8977-8982.
11. Javeshghani, S., Zakikhani, M., Austin, S., Bazile, M., Blouin, M.J., Topisirovic, I., St-Pierre, J. and Pollak, M.N. (2012). Carbon source and myc expression influence the antiproliferative actions of metformin. *Cancer Research* 72 (23), 6257-6267.
12. Julien S, Dube N, Hardy S, and Tremblay ML. (2011). Inside the human cancer phosphatome, *Nature Reviews Cancer*: 11:1
13. Eichner LJ, Perry MC, Dufour CR, Bertos N, Park M, St-Pierre J, and Giguère V. (2010). miR-378 mediates metabolic shift in breast cancer cells via the PGC-1β/ERRγ transcriptional pathway. *Cell Metab*: Oct 6;12(4):352-61
14. Pollak M. (2010). Beyond steroid hormones: the new cancer endocrinology. *Lancet Oncol* : 11: 501-2
15. Hardy, S., Wong, N.N., Muller, W.J., Park, M., and Tremblay, M. (2010). Overexpression of Protein Tyrosine Phosphatase PRL2 correlates with breast tumour formation and progression. *Cancer Res*. 70, 8959-8967.
16. Dowling RJ, Topisirovic I, Alain T, Bidinosti M, Fonseca BD, Petroulakis E, Wang X, Larsson O, Selvaraj A, Liu Y, Kozma SC, Thomas G, Sonenberg N. (2010). mTORC1-mediated cell proliferation, but not cell growth, controlled by the 4E-BPs. *Science*: May 28;328(5982):1172-6

The Terry Fox New Frontiers Program in Pre-clinical Models and Therapeutic Targets for Metastatic Breast Disease *(2009-2014)*

Investigators: William Muller, Vincent Giguere, Morag Park, Peter Siegel, Mike Hallett, McGill University

Scientific Summary: The projects within this program revolve around how interactions between breast cancers cells and their tissue microenvironment impact their ability to colonize other tissue sites.

Project one (Muller Lab) relies on a unique transgenic mouse model that expresses activated ErbB2/Neu under the transcriptional control of the endogenous *erbB2* promoter (ErbB-2KI model). Like *erbB2* expressing human breast cancers, mammary tumour progression in this model is associated with the selective amplification of the *erbB2* locus. Using gene expression profiling, we have established that tumours derived from ErbB2-KI model closely resemble ErbB2 positive class of human breast cancers. These analyses also showed that Wnt-catenin pathway was engaged in these tumours. Using a RNA interference approach we further demonstrated that down-regulation of catenin function had a profound effect on both tumour growth and metastasis in cell lines derived from both mouse and human ErbB2 positive breast cancer.

Project two (Giguere Lab) investigates how retinoic acid influences the initiation and progression of breast cancer induced by erbB2. Retinoic acid, the active metabolite of vitamin A, is a known anti-cancer agent that promotes cell differentiation and inhibits cell proliferation. Unexpectedly and most strikingly, we observed that RAR? knock-out mice are completely resistant to mammary tumours induced by activated ErbB2 when expressed from its own promoter. We observed that the delay in tumour initiation and reduction in tumour growth rate in RAR?–null mice are dictated by the absence of RAR? in the stromal micro-environment. Indeed, loss of RAR? in epithelia has no significant impact on tumour growth when introduced in the stromal compartment of wild-type mice. Our current work is to determine the specificity of the RAR isoform/oncogene interaction in mammary gland tumourigenesis, identify transcriptional regulatory networks in the stromal and tumour compartments involved in the oncogenic process and explore their relevance to human breast cancer and to investigate the role of non-canonical RA signalling pathways possibly involved in mammary gland tumourigenesis.

Project three (Siegel Lab) has focused on identifying tumour intrinsic and microenvironment-regulated factors that control the ability of breast cancer cells to grow in the liver. To accomplish this, we have performed *in vivo* selection using 4T1 breast cancer cells to identify genes that are associated with the liver metastatic phenotype. Coincident with the loss of numerous tight-junctional proteins, we observe that claudin-2 is specifically over expressed in highly liver-aggressive, *in vivo* selected breast cancer cells and is highly expressed in liver metastases from breast cancer patients. We further demonstrate that claudin-2 is both necessary and sufficient for the ability of 4T1 breast cancer cells to colonize and grow in the liver. Together, these results uncover novel roles for claudin-2 in promoting breast cancer adhesion to the extracellular matrix, through enhancing the formation of functional integrin receptors, and define its importance during breast cancer metastasis to the liver.

Project four (Park Lab) is focused on identifying the key molecular events involved in the induction of the basal sub-type of breast cancer. Since many triple-negative breast cancers express a non-functional p53, the MMTV-*Met* model was inter-crossed with mice bearing a conditional deletion of *Trp53* in the mammary gland (MMTV-*Met* x $Trp53^{F2\text{-}10}$ x MMTV-*Cre).* In addition to a significantly increased tumour penetrance and decreased latency, loss of *Trp53* in combination with Met^{Mu} transgene expression leads to the formation of tumours with predominantly EMT morphology. Thus, these tumours display important hallmarks of claudin-low breast cancer and present a unique model to identify novel therapeutic targets for the treatment of this sub-type of breast cancer.

List of Key Publications:

1. Schade, B, Lesurf, R. Sanguin-Gendreau, V. Millar, E. Zardawi, S. W. , Lopez-Knowles , E. , Sutherland, R.L., O'Toole, S. A, Kahn, M.Hallett, M. and William J. Muller. (2013) b-catenin signaling is a critical event in ErbB2 mammary tumour progression. *Cancer Res*. 73, 4474-4487 PMID 23720052

2. Dupuy, F., Griss, T., Blagih, J., Bridon, G., Avizonis, D., Ling, C., Dong, Z., Siwak, D., Annis, M. G., Mills, G.B., Muller, W.J., Siegel, P.M., and Jones, R. G. (2013). LKB1 is a central regulator of tumour initiation and pro-growth metabolism in ErbB2-mediated breast cancer. *Cancer and Metabolism* (in press)

3. Karin, S., Creedon, H., Patel., H., Carragher, N. G., Morton, J., Muller, W., Evans, T., Gusterson, B., Samson, O. and Bruton, V. (2013). Dasatnib inhibits tumour development in a genetically engineered mouse model. *J. Pathol.* 230. 430-44

4. Knight, J.F., Lesurf, R., Zuo, D., Zhao, H., Davis, R.,Pylypenko, I., Saleh, S. , Pinnaduwage, D., Mulligan, A.M., Cardiff, R.D., Gregg, J., Andrulis, I.L., Hallett, M.,

Muller, W.J., and Park, M. (2013). Met synergizes with p53 loss to induce mammary tumours that possess features of Claudin-low breast cancer. *PNAS (USA)* 110 E1301-1310. PMID23509284

5. *Liu X, Nugoli M, Laferriere J, Saleh SM, Rodrigue-Gervais IG, Saleh M, Park M, Hallett MT, Muller WJ & Giguere V. Stromal retinoic acid receptor b promotes mammary gland tumourigenesis. *Proc. Natl. Acad Sci:* USA 108(2): 774-779 (2011).

6. *Tabariès S, Dong Z, Annis MG, Omeroglu A, Pepin F, Ouellet V, Russo C, Hassanain M, Metrakos P, Diaz Z, Basik M, Bertos N, Park M, Guettier C, Adam R, Hallett M & Siegel PM. Claudin-2 is selectively enriched in and promotes the formation of breast cancer liver metastases through engagement of integrin complexes. *Oncogene*: 30(11):1318-1328 (2011).

7. *Tabariès, S. and Siegel, P.M. Breast Cancer Liver Metastasis. In Pnina Brodt (Ed), *Liver Metastasis: Biology and Clinical Management, Cancer Metastasis – Biology and Treatment*. DOI 10.1007/978-94-007-0292-9_10. *Springer Science and Business Media* B.V (2011).

8. Huck L, Pontier S, Zou DM & Muller WJ. ?1-integrin is dispensable for the induction of ErbB2 mammary tumours but plays a critical role in the metastatic progression. *Proc. Natl. Acad Sci: USA*: 107(35): 15559-15564 (2010).

9. *Ranger JJ, Levy DE, Shahalizadeh S, Hallett M & Muller WJ. Identification of a Stat3-dependent transcription regulatory network involved in metastatic progression. *Cancer Research:* 69(17):6823-6830 (2009).

10. *Siegel PM & Muller WJ. Transcription regulatory networks in mammary epithelial development and tumourigenesis. *Oncogene:* 29(19): 2753-2759 (2010).

11. *Fathers K, Monast A, Rodrigues S, Zuo D, Cardiff R & Park M Crkll transgene induces atypical mammary gland development and tumourigenesis. *Am. J. Pathol:* 176(1):446-60 (2010).

12. Ponzo MG & Park M(2010). The Met receptor tyrosine kinase and basal breast cancer. *Cell Cycle* 9(6):1043-1050 (2010).

13. Bertos NR & Park M . Tumours and their Microenvironments. In: Wang E (ed.) *Cancer Systems Biology*, CRC Press (2010). (ISBN: 978-1-4398-1185-6).

14. *Ponzo MG, Lesurf R, Petkiewicza S, O'Malley FP, Pinnaduwage D, Andrulis IL, Bull SB, Chughtai N, Zuo D, Souleimanova M, Germain D, Omeroglu A, Cardiff RD, Hallett M & Park M. Met induces mammary tumours with diverse histologies and is associated with poor outcome and human basal breast cancer. *PNAS:* 106, 12903-08 (2009).

15. *Finak G, Laferrière J, Hallett, M & Park M.The tumour microenvironment: a new tool to predict breast cancer outcome. Invited News in *Médecine / Sciences* 25-439-441(2009).

16. *Deblois G, Hall J, Perry M-C, Laganiere J, Ghahremani M, Park M, Hallett M & Giguere V. Genome-wide identification of direct target genes specifies ERR? signaling as a determinant of breast cancer heterogeneity. *Cancer Research* 69, 6149-57 (2009).

Asterisk (*) indicates publications that have multiple team members as authors.

The Terry Fox New Frontiers Program Project Grant in the Genetic Analysis of Signaling Pathways for Vascular Development and Tumour Angiogenesis *(2010-2015)*

Investigators: Andras Nagy, Anthony Pawson, Jeff Wrana, Susan Quaggin; Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Hao Ding; University of Manitoba, Janet Rossant; Hospital for Sick Children

Scientific Summary: Anti-angiogenic therapy is one of the most promising cancer treatments. It prevents the formation of new blood vessels and thereby hinders tumours from growing. This treatment, however, comes with a long list of severe side effects, including high blood pressure and kidney failure, which limits the full utilization of this powerful approach to fighting cancer. In this program project, we join the expertise of six laboratories to overcome these limitations.

- The Nagy Lab is committed to obtain mechanistic insights into the broad range of consequences of anti-angiogenic therapies on normal physiology including the immune system.
- The Quaggin Lab focuses on finding ways to protect the kidney and other organs from damage during anti-angiogenic therapy.
- The Rossant Lab uses stem cells to increase the quality of blood vessels so that the delivery of chemotherapy can be enhanced. They also develop robust cell-based screening tools to test new anti-angiogenic therapies.
- The Wrana Lab has developed a system to measure the movement of cancer cells. They use this to define novel pathways mediating cancer metastasis via stromal tumour interactions.
- The Ding Lab investigates how the PDGF gene, that plays an important role in vessel formation, is involved in medulloblastomas, the most common type of brain tumour in children.
- The Pawson Lab studies how disruptions of cell-cell interactions can cause cancer.
- There are two core facilities serving our laboratories; one maintains and distributes genetically modified mouse lines for cancer modeling and the other provides high throughput screens.

List of Key Publications:

1. Adipose Vascular Endothelial Growth Factor Regulates Metabolic Homeostasis through Angiogenesis. Sung HK, Doh KO, Son JE, Park JG, Bae Y, Choi S, Nelson SML, Cowling R, Nagy K, Michael IP, Koh GY, Adamson SL, Pawson A, Nagy A. (2013) *Cell Metabolism*. Volume 17, Issue 1, 61-72, 8 January 2013. PMID: 23312284

2. Soluble FLT1 Binds Lipid Microdomains in Podocytes to Control Cell Morphology and Glomerular Barrier Function. Jin J, Sison K, Li C, Tian R, Wnuk M, Sung HK, Jeansson M, Zhang C, Tucholska M, Jones N, Kerjaschki D, Shibuya M, Fantus IG, Nagy A, Gerber HP, Ferrara N, Pawson T, Quaggin SE. (21012) *Cell.* 2012 Oct 12;151(2):384-99. PMID: 23063127

3. Interaction domains of Sos1/Grb2 are finely tuned for cooperative control of embryonic stem cell fate. Findlay GM, Smith MJ, Lanner F, Hsiung MS, Gish GD, Petsalaki E, Cockburn K, Kaneko T, Huang H, Bagshaw RD, Ketela T, Tucholska M, Taylor L, Bowtell DD, Moffat J, Ikura M, Li SS, Sidhu SS, Rossant J, Pawson T. *Cell.* 2013 Feb 28;152(5):1008-20. PMID:23452850

4. The adaptor protein Grb2 is not essential for the establishment of the glomerular filtration barrier. Bisson N, Ruston J, Jeansson M, Vanderlaan R, Hardy WR, Du J, Hussein SM, Coward RJ, Quaggin SE, Pawson T. *PLoS One*. 2012;7(11):e50996. Epub 2012 Nov 30. PMID:23226445

5. Exosomes mediate stromal mobilization of autocrine Wnt-PCP signaling in breast cancer cell *mtility*. Luga, V.*, Zhang, L.*, Viloria-Petit, A., Ogunjimi, A., Inanlou, M., Chiu, E., Nasser Hosein, A., Buchanan, M., Basik, M. and Wrana, J.L. (2012). *Cell*, 151, 1542-1556. PMID: 23260141

The Terry Fox New Frontiers Program Project Grant in Molecular and Cellular Differentiation: New Targets and Treatments *(2009-2014)*

Investigators: Christopher Paige, Norman Iscove, John Dick, Ben Neel, Juan Carlos Zuniga-Pflucker, OCI, UHN; Tak Mak and Pam Ohashi, OCI, UHN

Scientific Summary: Fully functional mature cells differentiate from progenitors through a series of stages regulated by genes and proteins. Our group studies both the normal process of differentiation and the changes that occur when malignancies arise. We define the molecular differences that serve to drive the transformation and progression of cancer cells. Based on this information we develop and use novel technologies to find targets for therapeutic intervention. We study both solid and dispersed cancers using both mouse and human models with a particular emphasis on ovarian cancer, as an example of solid tumours, and leukemia. We also use the latest understanding of the cells and cytokines which drive immunity to develop novel protocols to harness the power of the immune system to recognize and eliminate cancer cells.

List of Key Publicatons:

1. Frelin C, Herrington R, Janmohamed S, Barbara M, Tran G, Paige CJ, Benveniste P, Zuñiga-Pflücker J-C, Souabni A, Busslinger M, Iscove NN. An unexpected role for Gata3 in regulating the self-renewal of long-term hematopoietic stem cells. *Nature Immunology*, in press 2013.

2. Wei LZ, Xu Y, Nelles ME, Furlonger C, Wang JCM, Di Grappa MA, Khokha R, Medin JA, Paige CJ. Localized interleukin-12 delivery for immunotherapy of solid Tumours. Localized interleukin-12 delivery for immunotherapy of solid Tumours. *J. Cell Mol Med.*, in press 2013

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The Terry Fox New Frontiers Program Project Grant in Genomic Determinants of Childhood Leukemia *(2010-2013)*

Investigators: Daniel Sinnett, Denise Avard, Bartha Knoppers, McGill University, Philip Awadalla, Maja Krajinovic, Caroline Laverdière, Marie-Hélène Roy-Gagnon, University of Montreal; Tomi Pastinen, Alexandre Montpetit; McGill University; Sherif Abou Elela, University of Sherbrooke; Sheryl Arrowsmith, OCI, UofT

Scientific Summary: Our team is using a combination of modern genomic approaches to provide an in-depth characterization of the childhood Acute Lymphoblastic Leukemia (ALL) genome, transcriptome, and methylome. Our aim is to create a detailed catalogue of the sequence, structural and epigenetic variations in childhood ALL to better understand how they affect biological pathways deregulated in disease, and to explore mechanistic links to disease susceptibility, disease onset and progression, and treatment responses. These findings will lead to the development of new research and clinical tools that will improve detection, diagnosis and treatment of children with ALL. An important component of this project is also to ensure that these developments are appropriately and efficiently translated to improve patient health and health care services.

Specific Goals:

- To identify sequence and structural variants in childhood ALL genomes using a unique quartet design (matched normal-tumoural patient samples and both parents), and next-generation whole-exome sequencing and high-density SNP genotyping;
- To investigate epigenetic and gene-expression changes in childhood leukemia genomes through RNA-sequencing, genome-wide allele-specific expression assays, and high-throughput DNA methylation profiling;
- To explore the impact of selected variants (and associated genes) on disease susceptibility and disease outcomes and investigate their functional significance using high-throughput phenotypic assays leukemia cell lines;
- To translate our genetic discoveries into appropriate health care, policy and services.

List of Key Publications:

1. Spinella JF, Saillour V, Bourgey M, Alter A, Larivière M, Healy J, Richer C, Busche S, Montpetit A, Ge B, Pastinen T, Sinnett D. The genomic landscape of childhood pre-B acute lymphoblastic leukemia. (submitted).

2. Healy J, Saillour V, Bourgey M, Alter A, Larivière M, Spinella JF, Richer C, Busche S, Ge B, Montpetit A, Awadalla P, Pastinen T, Sinnett D. Whole-exome sequencing of a rare case of familial childhood acute lymphoblastic leukemia. (Submitted)

3. Hussin J, Sinnett D, Casals F, Idaghdour Y, Bruat V, Saillour V*, Healy J, Grenier JC, de Malliard T, Busche S, Spinella JF, Larivière M*, Gibson G, Andersson A, Holmfeldt L, Ma J, Wei L, Zhang J, Andelfinger G, Downing JR, Mullighan CG, Awadalla P. Rare allelic forms of PRDM9 associated with childhood leukemogenesis. *Genome Res*. 2013 Mar;23(3):419-30.

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The Terry Fox New Frontiers Program Project Grant in Nanoparticle-Enhanced Photoacoustic Imaging for Cancer Localization and Therapeutic Guidance *(2013-2016)*

Investigators: Brian Wilson, Princess Margaret Cancer Center (Project Leader and Sub-project 3 Leader); Stuart Foster, Sunnybrook Research Institute (Sub-project 1 Leader); Gang Zheng, UHN (Sub-project 2 Leader); John Trachtenberg, Ralph daCosta, Princess Margaret Cancer Centre; Norman Marcon, Cathy Streutker, Maria Cirocco, St. Michael's Hospital; Theo van der Kwast, Robert Weersink, UHN; Linda Sugar, Masoom Haider, Sunnybrook Research Institute

Scientific Summary: This new project aims to develop a novel technology platform that will be applied to address two significant unmet needs in cancer control. The first component of the platform is photoacoustic imaging that combines the molecular specificity of light with the deep imaging capability of ultrasound. The second is the use of recently discovered all-organic nanoparticles that have an exceptional range of properties for imaging and therapeutics. Here, they will be used primarily for their unique photoacoustic imaging characteristics. The first unmet need is to treat patients with low/intermediate-risk "focal" prostate cancer by local destruction of the dominant ("index") lesion with minimal risk to normal tissues. This approach will build on developmental work and clinical trials done to date [1] using laser photothermal therapy, in which one or more fine optical fibers are used to deliver near-infrared light into the target region of the prostate to destroy the tissue by heating. The nanoparticle enhanced photoacoustic imaging platform will be used to improve the efficacy and safety of this new therapeutic paradigm and also to enable its wide dissemination and adoption into clinical practice. The second unmet need is to detect high-grade dysplasia in patients with Barrett's Esophagus and to assess whether or not there is submucosal invasion [2], so that, where appropriate, minimally-invasive endoscopic mucosal resection can be performed, reducing the need for esophagectomy and its associated morbidity. In both applications, the long term goal is to change the balance between achieving effective Tumour control and the side effects of radial therapies that significantly impact quality of life. The project comprises three subprojects, as follows:

Sub-project one "Photoacoustic Imaging Technology" focuses on the design, fabrication and bench-top performance testing and optimization of hardware and software for photoacoustic imaging. The technology is based on previous work by Dr Foster's group on high-frequency (and hence high resolution) ultrasound imaging, which is currently entering multicenter clinical trials for prostate cancer detection, extended this to photoacoustic mode by the addition of a multi-wavelength pulsed laser [3]. Using a common core instrument, two different "probes" will be developed. The first is a transrectal device for imaging of the prostate, while the second will be for endoscopic use, particularly for imaging the esophageal wall. Upon bench-top optimization, these two probes will be validated in animal models *in vivo* before translating to the first-in-human studies in Sub-project 3.

Sub-project two "Porphysomes for Photoacoustic Imaging" comprises further development and optimization of porphysomes. Two different forms of porphysome will be utilized. The first, pyro-porphysomes, are based on our original discovery [4], and will be used in both prostate cancer and Barrett's Esophagus as photoacoustic image-contrast agents. The second, J-porphysomes [publication in preparation], were conceived as a direct result of this TFRI project and will enable real-time 3-D imaging of the temperature distribution during photothermal treatment of focal prostate cancer, thereby enabling optimal and personalized treatment delivery. Both untargeted and biomarker-targeted versions of the porphysomes will be developed, depending on the clinical objective. For each porphysome type, we will optimize the synthesis and test their performance both in phantoms and in preclinical Tumour models *in vivo*. This will then be followed by scale-up, toxicity testing and gmp (good manufacturing practice) preparation of the nanoparticles for the first-in-human studies. Subproject 2 "First-in-Human Studies of PAI and Porphysomes in PCa and GI" comprises a series of small-scale studies in patients, piggybacking on either standard clinical practice or on-going clinical trials, with the intent to obtain first-in-human data on safety, technical feasibility and performance of the different photoacoustic/porphysome platforms. Four separate studies are proposed relating to prostate cancer: Tumour delineation using either intrinsic (i.e. contrast-free) or untargeted porphysome-enhanced photoacoustic imaging in combination with multiparametric MRI [5] to plan photothermal treatment; intrinsic photoacoustic imaging post treatment to assess the treatment response; thermal mapping using J-porphysomes during treatment; and intrinsic photoacoustic imaging to monitor the treatment response during treatment.

In the gastrointestinal track, we will first use the transrectal system (developed primarily for prostate) to assess photoacoustic imaging of rectal cancer; then test photoacoustic imaging, with and without pryro-porphysome contrast, to identify high-grade dysplasia in Barrett's Esophagus in *ex vivo* (endoscopic mucosal resection) tissues; and finally perform *in vivo* endoscopic photoacoustic imaging in the GI tract, particularly in Barrett's patients undergoing surveillance endoscopy. These studies will inform subsequent definitive clinical trials of the new technologies.

References: Since this project only started in July 2013, no TFRI-supported papers have been published to date. The references below are among key prior papers by our groups that have enabled this project.

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The Terry Fox New Frontiers Program Project Grant in Hypoxia in Tumours: Clinical and Experimental Studies *(2009-2014)*

Investigators: Robert Bristow, Bradly Wouters, Michael Milosevic, Ivan Yeung, Marianne Koritzinsky, Richard Hill, David Headly, OCI, PMCC; Anthony Fyles, PMCC; David Jaffray, OCI, UHN; Christine Allen, UofT

Scientific Summary: The overall hypothesis of our program is that the dynamic nature of the tumour microenvironment is an important contributor to the development, behaviour and prognosis of many solid cancers. Tumour cells sense and respond to their microenvironment through distinct biological pathways that contribute to aggressive tumour behaviour and treatment response. The current program consists of four funded and two leveraged projects with clinical, pre-clinical and basic science objectives. We are characterizing the nature and importance of the tumour microenvironment in cervix, prostate, and pancreatic tumours in clinical trials of novel targeted agents through measurements of genomics, hypoxia, IFP, perfusion, metabolism and energy status using a range of 2D, 3D and 4D imaging methodologies. These efforts are supported by the development of advanced 4D imaging and analysis techniques with a focus on molecule transport in tumours. The clinical studies are directly integrated with fundamental studies into the nature of oxygen sensing in tumours and the influence of hypoxia on signalling pathways that influence important tumour phenotypes including cellular metabolism, DNA repair, stemness, and metastasis.

Recent Discoveries and Accomplishments:

- Developed an approach to handle complex biomarker information within solid tumours using combination of IHC, genomic and imaging data.
- Over 400 patients have been accrued to clinical trials in prostate and cervix cancer in which information regarding hypoxia has helped to define new cancer therapies.
- Showed that hypoxia is associated with biochemical relapse after radiotherapy for prostate cancer and is an independent prognostic marker with genetic instability.
- IFP is a biomarker of chemo-radiation response in cervix cancer.
- Hedgehog signalling is associated with hypoxia in cervix cancer biopsies.
- Developed novel approaches to map altered trans-capillary and interstitial fluid dynamics in solid tumours that which underlie high IFP, the EPR effect and impaired delivery and transport of nutrients and therapeutic agents using scalable liposomal imaging contrast agents.
- Developed a novel method to assess tumour perfusion with a reduction of CT dose to patients by five to ten fold. This reduction will increase safety and enable longitudinal studies during treatment
- Showed that hypoxic cells can acquire deficiency in DNA repair (decreased HR, MMR and BER) due to altered protein translation of DNA repair genes as the basis of genetic instability.
- Repair-deficient hypoxic cells may now be considered as targets of novel molecular targeted agents, such as PARPi inhibitors
- Identified hypoxic regulation of mTOR signalling and metabolic activity in cancer and demonstrated the potential of targeting this pathway to improve radiation response.
- Showed that activation of the unfolded protein response mediates hypoxia tolerance through regulation of autophagy.
- Showed metabolic remodeling of the tumour microenvironment by metformin can decreased tumour radioresistance in vivo and in clinical cohorts treated with radiotherapy.
- Demonstrated the importance of mRNA translational control in mediating changes in gene expression during hypoxia.
- Showed that cyclic hypoxic enhances lymphatic metastasis in orthotopic cervix cancer models including patient-derived xenografts (simulates clinical findings).
- Demonstrated that blocking the action of the hypoxia induced gene VEGF-C and its receptor VEGFR3 prevents hypoxia induced increase in metastasis.

- Demonstrated a highly significant association between hypoxia and aggressive biology in patient-derived pancreas cancer xenografts. This suggests that hypoxia is major adverse prognostic feature of pancreas cancer, and supports testing novel agents to target hypoxia.

List of Key Publications:

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2. Chan N, Pires I, Benkocova Z , Coackley C , Bhogal N , Lakshman M , Gottipati P , Oliver J, Helleday T , Hammond E, Bristow RGF. Contextual Synthetic Lethality of Cancer Cell Kill Based on the Tumour Microenvironment. *Cancer Research*: 70(20):8045-54 (2010).

3. Kumareswaran R, Ludkovski O, Meng A, Sykes J, Pintilie M, and Bristow R. Chronic hypoxia compromises DNA double-strand break repair to drive genetic instability. *J Cell Science*: 125:189-99 (2012).

4. Pintilie M, Iakovlev V, Fyles A, Hedley D, Milosevic M, Hill RP. Heterogeneity and power in clinical biomarker studies. *J Clin. Oncol*: 27:1517-1521 (2009).

5. Rouschop KM., van den Beucken T, Dubois L, Niessen H, Bussink J, Savelkouls K, Keulers T, Mujcic H, Landuyt W, Voncken JW, Lambin P, van der Kogel AJ, Koritzinsky M, Wouters BG. The unfolded protein response protects human tumour cells during hypoxia through regulation of the autophagy genes MAP1LC3B and ATG5.*J Clin Invest*: 120(1): p.127-41 (2010).

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12. Chaudary N, Mujcic H, Wouters BG, Hill RP Hypoxia and metastasis in an orthotopic cervix cancer xenograft model.*Radiother Oncol.* 2013 Jul 12 (In Press)

13. Cojocari D, Vellanki RN, Sit B, Uehling D, Koritzinsky M, Wouters BG. New small molecule inhibitors of UPR activation demonstrate that PERK, but not IRE1α signaling is essential for promoting adaptation and survival to hypoxia. *Radiother Oncol.* 2013 Jul 3 (In Press)

14. Rouschop KM, Dubois LJ, Keulers TG, van den Beucken T, Lambin P, Bussink J, van der Kogel AJ, Koritzinsky M, Wouters BG. PERK/eIF2α signaling protects therapy resistant hypoxic cells through induction of glutathione synthesis and protection against ROS. *Proc Natl Acad Sci U S A.* 19;110(12):4622-7, (2013).

15. Tamara Marie-Egyptienne D, Lohse I, Hill RP Cancer stem cells, the epithelial to mesenchymal transition (EMT) and radioresistance: Potential role of hypoxia. *Cancer Lett.* 2013 (In Press)

STP collaboration with the Centre for Drug Research and Development (CDRD)

Terry Fox Research Institute Translational Cancer Research Project (2013-2015)

Investigators: John Babcook, CDRD; Rob Rottapel, Ben Neel, Brad Wouters, OCI; David Andrews, McMaster University; Peter Dirks, Hospital for Sick Children; Daniel Durocher, Frank Sicheri, Samuel Lunenfeld Research Institute, Mount Sinai Hospital; Jason Moffat, Sachdev Sidhu, UofT

Scientific Summary: The Selective Therapies Program (STP) is a translational program whose objective is to identify novel cancer targets for which new anti-cancer therapeutics can be developed with heightened selective properties. The STP has been able to identify novel cancer targets using high-throughput RNA interference (RNAi) screening technologies and new high-throughput screening strategies. Dr. Sidhu has led the development of novel synthetic antibodies to promising cancer drug targets identified by the STP. To further exploit the therapeutic potential of these novel targets and antibodies, the Centre for Drug Research and Development (CDRD) will select antibodies generated by the STP and conjugate their novel, potent cytotoxins to generate antibody-drug conjugates (ADCs). These ADCs will be assessed for their ability to specifically deliver the toxin payloads and kill target-expressing tumour cells. Lead ADC candidates will then be selected for further therapeutic development.

Development of new treatment and biomarker for hepatocellular carcinoma: From woodchuck to human

NSC-TFRI International Collaborative Research (2013-2016)

Investigators: John Bell, OHRI; Pei-Jer Chen, National Taiwan University College of Medicine

Scientific Summary: Lack of sensitive biomarkers for timing diagnosis and effective therapeutics for advanced tumours are the two main reasons for the poor outcome of hepatocellular carcinomas (HCC). Therefore, there is a pressing demand to develop new diagnosis and treatment strategies for HCC. Chronic hepatitis B virus (HBV) infection is one of the major causes of HCC and the woodchuck (*Marmota monax*) chronically infected with woodchuck hepatitis virus (WHV), a virus with high similarity to human HBV, recapitulates the complex liver milieu and natural course from chronic HBV infection to HCC. It represents, and has been used, as an ideal preclinical model for HBV-related translational studies. Despite the approval of the molecular targeted agent, sorafenib, for advanced HCC treatment, its efficacy has been modest. New regimens, such as oncolytic viruses, are promising anti-cancer agents that deserve further investigation to optimizing efficacy and safety in the relevant woodchuck model.
Therefore, the objectives of this project are:

- To adapt oncolytic virus-based therapeutics based on vaccinia virus or rhabdovirus platforms for testing against liver cancer in the woodchuck hepatitis B model;
- To use surgical explants from woodchuck or human HCC subjects to study their susceptibilities to oncolytic virus infection and to identify transcriptional or genomic markers that predict animal/patient HCC permissiveness for oncolytic virus infection and treatment responses;
- To develop unique viral integration cellular junction DNA as a biomarker for follow-up of HCC growth and treatment in woodchucks.

List of Key Publications:

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Development of 2-[^{18}F]fluoro-2-deoxy-D-galactose as a new molecular imaging probe for hepatocellular carcinoma diagnosis

NSC-TFRI International Collaborative Research (2013-2016)

Investigators: François Benard, BC Cancer Agency; Kai-Yuan Tzen, National Taiwan University Hospital

Scientific Summary: Liver cancer is a major cause of death among patients of east or southeast asian descent, as well as other population groups, notably in central and west Africa. Diagnosis of liver cancer requires a combination of several imaging techniques and biopsies. Despite this, diagnosis can remain inconclusive or difficult to establish in patients at risk for liver cancer.

The purpose of this joint Taiwanese / Canadian research project is to evaluate novel imaging methods developed to diagnose the most common form of liver cancer, hepatocellular carcinoma. We propose to use novel imaging probes that have been reported to bind to liver cancers but not benign liver lesions that can be confused with liver cancer. Three such imaging probes will be evaluated. 2-[^{18}F]-fluoro-2-deoxy-D-gulcose, called [^{18}F]FDG, is a radioactive sugar that is widely used for cancer imaging with a device called positron emission tomography, or PET scans. We already know that [^{18}F]FDG cannot detect some liver cancers that are slow growing. 2-[^{18}F]Fluoro-2-deoxy-D-galactose([^{18}F]FDGal), another radioactive sugar, has been recently reported to be highly effective at detecting liver cancer. [^{18}F]Fluorocholine ([^{18}F]FCH), another molecule, is currently being evaluated in Taiwan and other jurisdictions for this purpose. In 2010, a French researcher reported 80-90% detection rate by using [^{18}F]FCH alone or in combination with [^{18}F]FDG. In 2011, a Danish researcher reported an even better result by using [^{18}F]FDGal alone.

The Taiwan group will compare [^{18}F]FCH and [^{18}F]FDGal. The Canadian (Vancouver) group will compare [^{18}F]FDGal and [^{18}F]FDG, which could be complementary to each other. Both groups will evaluate 50 patients each over a period of three years. The results will be correlated with those of biopsies and clinical follow-up. Having two patient groups will allow the researchers to compare two strategies, while minimizing the number of diagnostic tests that research participants will have to undergo to evaluate the best diagnostic strategy. After the completion of these two trials, we will compare the results with another on going multi-centre trial now already on schedule in Taiwan by 10 medical centres using [^{18}F]FCH vs. [^{18}F]FDG.

This study will provide valuable data on whether these imaging agents can successfully differentiate malignant liver lesions from benign ones. It will also provide information about whether these imaging agents can successfully assess whether the cancer has spread outside the liver. It will provide data that will allow physicians to determine the optimal imaging protocol to properly diagnose liver cancer.

Modeling and therapeutic targeting of the clinical and genetic diversity of glioblastoma

Terry Fox Research Institute Translational Cancer Research Project (2012-2017)

Investigators: Gregory Cairncross, Stephen Robbins, Samuel Weiss, University of Calgary; David Kaplan, UofT; Warren Mason, Queen's University; Marco Marra, UBC

Scientific Summary: Glioblastoma (GBM) is a deadly brain cancer that has eluded major treatment advances. While all agree that new therapies for GBM are needed, there is no consensus on how best to find them. With this project we employ a unique collection of cell lines established from GBM. These lines, referred to as *brain tumour initiating cells* (BTICs), capture and retain the major genetic alterations that are present in the tumours from which they were derived, in addition to maintaining many of the histological features of the parent tumour when grown *in vivo*. This cell-based model system now provides our team at the universities of British Columbia, Calgary and Toronto with the foundation for an innovative drug discovery and genome-sequencing program with real potential for rapid clinical translation. Our experimental strategy begins with BTIC lines as a research tool for drug and target discovery and ends with new therapeutics in early phase human testing in molecularly defined subpopulations of GBM, via a collaboration with the NCIC Clinical Trials Group and its many participating Canadian centres. Our approach, which combines a superior model system with high-throughput drug screening and genomics technologies, holds great promise. Our singular objective is the discovery of new drug therapies for GBM within five years that will improve tumour control and quality of life for patients with this disease. This project will also ensure that specialized laboratory models of GBM are in hand to support future drug discovery.

Specific Aims:

- High-throughput screening of toolkit, NIH, and kinase inhibitor libraries against a panel of BTICs to enable rapid identification of targeted drug therapies for GBM.
- Genome and transcriptome sequencing of BTICs, their parent tumours, and normal DNA to enable the discovery of new drug targets, as well as correlate genotype with drug response.
- Continued establishment of BTIC lines from common and rare types of glioma.
- Pre-clinical testing of promising compounds *in vivo*.
- Clinical trials of promising compounds.

Multicentre Phase II study for international intraocular retinoblastoma

Terry Fox Research Institute Translational Cancer Research Project (2010-2013)

Investigators: Helen Chan, Brenda Gallie, Elise Heon, Helen Dimaras, Tony Panzarella, Hospital for Sick Children, OCI, and UofT

Scientific Summary: This multicentre Phase II study for bilateral intraocular retinoblastoma, using cyclosporine-circumvention of the multidrug resistance P-glycoprotein during carboplatin-etoposide-vincristine chemotherapy followed by focal laser therapy/cryotherapy consolidation, has been active since June 25, 2004. To date, we have treated in total 66 eyes in 40 patients: 18 patients at the Toronto site, 2 patients at the Vancouver site, 2 patients at the Montreal site, 14 patients at the Chennai/India site, 1 at the Singapore site, and 3 at the Santiago/Chile site. Recruitment is low because retinoblastoma is a very rare childhood eye cancer. On May 15, 2013, at 7.5 years after treating the first patient on the study, the toxicity is low (fever admissions 3.2%, sepsis 0%, blood transfusions 2.2%, platelet transfusions 8.1%). Efficacy is excellent. Of the 60 evaluable eyes in 36 evaluable patients, there are 43 remission eyes (43 eyes: 20 B eyes; 8 C eyes; 15 D eyes), and 17 relapsed eyes (16 eyes: 4 C eyes; 13 D eyes). Thirteen failed eyes (11 D eyes, 2 C eye) were treated with enucleation, 3 failed eyes (2 C eye, 1 D eye) were treated with radiation, and 1 failed eye (D eye) was treated with radiation followed by enucleation, at a median follow-up of 32 months with a range of 3 to 88 months follow-up. No Group B eye failed. Only one patient lost both eyes. The radiation rate is low (4 of the 36 patients have been radiated in one eye), and this is particularly important in the long term because of the very high lifelong risk of radiation-induced secondary malignancies in these germline RB1-mutated bilateral retinoblastoma patients. As of November 2007, to further reduce potential toxicity with this excellent efficacy, we received Institutional Research Board and Health Canada approval to reduce upfront chemotherapy cycles (from 6 to 4 cycles for severely affected eyes, and from 3 to 2 cycles for less affected eyes), since further cycles of the same protocol could be reused if clinically warranted during the focal laser therapy/cryotherapy consolidation period.

List of Key Publications:

1. Yousef YA, Halliday W, Dimaras H, Chan HSL, Héon E, Gallie BL. Subtenon Topotecan with Fibrin Sealant for Retinoblastoma Does Not Lead to Ocular Motility Complications. Canadian Journal of Ophthalmology, In Press 30 May, 2013.
2. Rootman DB, Gonzalez E, Mallipatna A, VandenHoven C, Hampton L, Dimaras H, Chan HSL, Gallie BL, Héon E. High-resolution spectral domain OCT in retinoblastoma: clinical and morphologic considerations. British Journal of Ophthalmology, 97:1, 59-65, 2013.
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4. Zhao J, Dimaras H, Massey C, Xu X, Huang D, Li B, Chan HSL, Gallie BL. Pre-enucleation chemotherapy for eyes severely affected by retinoblastoma masks risk of tumour extension and increases death from metastasis. *Journal of Clinical Oncology*, 29(7):845-51 (2011).
5. Dimaras H, Kimani K, Dimba EAO, Gronsdahl P, White A, Chan HSL, Gallie BL. Retinoblastoma. Refereed Lancet Seminar. In Press, Lancet, July 15 (2011).
6. Dimaras H, Rushlow D, Halliday W, Doyle J, Babyn P, Abella E, Williams J, Héon E, Gallie BL, Chan HSL. Using *RB1* mutations to assess minimal residual disease in metastatic retinoblastoma before stem cell transplant. *Translational Research*, 156(2):91-7 (2010).
7. Chan HSL, Gallie BL, Munier FL, Popovic MB. Chemotherapy for retinoblastoma. Refereed Chapter. In: Ophthalmic Oncology, Singh AD (ed): Ophthalmology Clinics of North America, Volume 18 (1): 55-63 (2005). Publisher Elsevier Saunders, Philadelphia.
8. Chan HSL, DeBoer G, Thiessen JJ, Budning A, Kingston JE, O'Brien JM, Koren G, Giesbrecht E, Haddad G, Verjee Z, Hungerford JL, Ling V, Gallie BL. Combining cyclosporin with chemotherapy effectively controls intraocular retinoblastoma (RB) without requiring radiation. *Clinical Cancer Res* 2:1499-1508 (1996).
9. Chan HSL, Haddad G, Thorner PS, DeBoer G, Lin YP, Ondrusek N, Yeger H, Ling V. P-glycoprotein expression as a predictor of the outcome of therapy for neuroblastoma. *N Engl J Med*; 325:1608-1614 (1991).

A randomized Phase II study of OGX-427 plus prednisone vs. prednisone alone in patients with chemotherapy-naive metastatic castration-resistant prostate cancer

Terry Fox Research Institute Translational Cancer Research Project (2010-2014)

Investigator: Kim N. Chi, BC Cancer Agency

Scientific Summary: Heat Shock Protein 27 (Hsp27) is a stress-activated, multi-functional chaperone protein highly expressed in cancer that regulates many cell signalling and survival pathways implicated in cancer progression. In prostate cancer models, Hsp27 complexes with androgen receptor (AR) and enhances transactivation of AR-regulated genes. OGX-427 is a second generation antisense that inhibits Hsp27 expression with *in vitro* and *in vivo* efficacy. Phase I studies have demonstrated tolerability and single agent activity.

Patients with Castration-Resistant Prostate Cancer (CRPC), no/minimal symptoms and any prior treatment other than chemotherapy were randomized 1:1 to receive Prednisone 5 mg PO BID or P with OGX-427 600 mg IV x 3 loading doses followed by 1000 mg IV weekly. The primary endpoint is the proportion of pts progression free (PSAWG 2 criteria) at 12 weeks. A 2-stage MinMax design (H0 = 5%, HA >20%, α=0.1, β=0.1) will enrol 32 pts total per arm and provide 70% power to detect the difference at a 0.10 1-sided significance. Secondary endpoints include PSA decline, measurable disease response, and circulating tumour cell (CTC) enumeration.

In the first stage analysis on the initial 44 patients randomized to the study, 50% of patients treated with OGX-427 had a PSA response, while only 20% of patients treated with prednisone had a PSA response. In patients with measurable disease, 44% of patients treated with OGX-427 had a partial or complete response, while 0% of patients on prednisone alone had an objective response. These preliminary data provide clinical support for the role of Hsp27 in AR signalling and as a therapeutic target for prostate cancer. Enrolment on this study has completed, and final data expected December 2013.

Improved assignment of best available therapy for patients with myelodysplasia and acute myeloid leukemia

Terry Fox Research Institute Translational Cancer Research Project (2012-2014)

Investigators: Stephen Couban, Dalhousie University; Aly Karsan, Stuart Peacock, Donna Hogge, Keith Humphries, BC Cancer Agency; Carl Hansen, UBC; John Shepherd, Leukemia/BMT Program of BC; Russell Greiner, University of Alberta; Lynn Savoie, Tom Baker Cancer Centre; Peter Chow-White, Simon Fraser University; Spencer Gibson, Versha Banerji, Cancer Care Manitoba; David Hedley, Mark Minden, Matthew Seftel, OCI; Tony Panzarella, Jeffrey Hoch, UofT; Guy Sauvageau, Josée Hébert, Brian Wilhelm, UdeM

Scientific Summary: The goal of TFRI MDS/AML Research Consortium is to improve outcomes in people with MDS/AML through validation of several promising complementary flow cytometric and genetic-based tests, to assign individual patients to the best available therapy. The TFRI MDS/AML Research Consortium will carry out a multi-institutional clinical study that will be sufficiently powered to determine whether one or more of the proposed tests are useful predictors of response to therapy, either alone or in combination with other tests. A major deliverable of this study will be one or more tests that will aid physicians in assigning people to the least toxic and most effective therapy. Another goal is to strengthen a nascent pan-Canadian collaboration in patients with MDS and AML.

The tests under study include:

- A flow cytometry assay for drug efflux targeting about 20% of newly diagnosed AML patients who exhibit chemotherapy refractory disease. The assay quantifies mitoxantrone efflux in the presence or absence of cyclosporine, an inhibitor of ABC transporter function, to give a measurement of chemotherapy refractoriness.
- A flow cytometry assay to monitor effects of molecularly targeted agents on AML, based on combinations of phosphospecific antibodies and phenotypic markers.
- Applying array comparative genomic hybridization (CGH) to the leukemia initiating cells enriched fraction of cells to improve the diagnostic efficacy of MDS patients leading to optimal therapy.
- Applying a novel high-throughput platform for quantitative miRNA profiling applicable to small cell numbers with the capacity to isolate by FACS discrete and highly purified leukemic cells.
- Applying RNA-seq transcriptome analysis to catalogue the mutations involved in normal karyotype AML.

List of Key Publications:

1. Kim HP, Bernard L, Berkowitz J, Nitta J & Hogge DE. A flow cytometry, mitoxantrone efflux-based assay for predicting chemotherapy refractoriness in newly-diagnosed acute myeloid leukemia. *Cytometry B Clin Cytom* 82B:283-294, 2012.

Pan-Canadian Colorectal Cancer Consortium (C4): Improving the health of Canadians by reducing colorectal cancer risk, by improving outcomes through earlier diagnosis and by improving therapeutic outcomes for those with more advanced disease

Terry Fox Research Institute Translational Cancer Research Project (2012-2014)

Investigators: Gerald Batist, Jewish General Hospital; Steven Gallinger, UHN

Scientific Summary: Colorectal cancer (CRC) is the most common malignancy of the gastrointestinal tract, and the second leading cause of cancer death among Canadians. The more advanced the disease is at the time of diagnosis, the greater the risk of metastasises. The main strategies proven to ameliorate the health of Canadians with colorectal cancer are 1) reducing risk, by improving outcomes through earlier diagnosis; and 2) increasing therapeutic responses for those with more advanced disease. The uptake of CRC screening in most Canadian provinces remains too low for the potential impacts of screening to be achieved across the population, even after recent efforts to increase screening rates. Although a variety of innovations in treatment have dramatically improved the outcome for patients with stage III and IV disease, the inevitable development of therapeutic resistance remains the major obstacle to improving survival.

The overall objective of this project is to establish a molecular-based approach to translational cancer care that will improve the outcome of CRC patients by:

- Increasing the impact of early diagnosis, decreasing mortality and the cost of managing CRC through targeted screening of families stratified by risk (Screening Axis).
- Improving the life expectancy and reducing the cost of the management of advanced CRC through the study of drug resistant metastatic disease and the development of a biomarker panel to predict drug resistance (Therapeutic Axis).

This will be achieved by creating the C4, a Canadian multidisciplinary and inter-institutional network. A major outcome of the C4 will be an integrated infrastructure for the development of a large-scale, molecular-based approach to translational cancer care for CRC. The C4 aims to use genetic data for the breadth of clinical challenges faced from the time of diagnosis to the time of treatment. The C4, supported by TFRI, will put Canada at the forefront of translational cancer research allowing us to establish a unique and high impact program.

List of Key Publications:

1. Identification of novel variants in colorectal cancer families by high-throughput exome sequencing. DeRycke MS, Gunawardena SR, Middha S, Asmann YW, Schaid DJ, McDonnell SK, Riska SM, Eckloff BW, Cunningham JM, Fridley BL, Serie DJ, Bamlet WR, Cicek MS, Jenkins MA, Duggan DJ, Buchanan D, Clendenning M, Haile RW, Woods MO, Gallinger SN, Casey G, Potter JD, Newcomb PA, Le Marchand L, Lindor NM, Thibodeau SN, Goode EL. *Cancer Epidemiol Biomarkers Prev*. 2013 Jul;22(7):1239-51. doi: 10.1158/1055-9965.EPI-12-1226. Epub 2013 May 1.

2. Identification of Lynch syndrome among patients with colorectal cancer. Moreira L, Balaguer F, Lindor N, de la Chapelle A, Hampel H, Aaltonen LA, Hopper JL, Le Marchand L, Gallinger S, Newcomb PA, Haile R, Thibodeau SN, Gunawardena S, Jenkins MA, Buchanan DD, Potter JD, Baron JA, Ahnen DJ, Moreno V, Andreu M, Ponz de Leon M, Rustgi AK, Castells A; EPICOLON Consortium. *JAMA*. 2012 Oct 17;308(15):1555-65. doi: 10.1001/jama.2012.13088.

3. Biopsies: next-generation biospecimens for tailoring therapy. Basik M, Aguilar-Mahecha A, Rousseau C, Diaz Z, Tejpar S, Spatz A, Greenwood CM, Batist G. *Nat Rev Clin Oncol*. 2013 Aug;10(8):437-50.

4. Next-generation biobanking of metastases to enable multidimensional molecular profiling in personalized medicine. Diaz Z, Aguilar-Mahecha A, Paquet ER, Basik M, Orain M, Camlioglu E, Constantin A, Benlimame N, Bachvarov D, Jannot G, Simard MJ, Chabot B, Gologan A, Klinck R, Gagnon-Kugler T, Lespérance B, Samson B, Kavan P, Alcindor T, Dalfen R, Lan C, Chabot C, Buchanan M, Przybytkowski E, Qureshi S, Rousseau C, Spatz A, Têtu B, Batist G. *Mod Pathol*. 2013 Jun 7.

5. Physician recruitment of patients to non-therapeutic oncology clinical trials: ethics revisited. Black L, Batist G, Avard D, Rousseau C, Diaz Z, Knoppers BM.*Front Pharmacol*. 2013;4:25.

In vivo validation of second generation nucleoside analogs

Terry Fox Research Institute Translational Cancer Research Project (2011-2013)

Investigators: Yvan Guindon, Institut de recherches cliniques de Montréal

Scientific Summary: Despite intensive research efforts, an effective treatment for pancreatic ductal adenocarcinoma (PDAC) still remains a major unmet medical need. With a dismal prognosis (one-year relative survival rate is 20%, and the five-year rate is 4%), this type of cancer is refractory to most existing chemotherapies. The current standard of care is Gemcitabine (GEM) alone and combination with 5-FU has shown very marginal benefit. Both drugs are nucleoside analogs (NA) acting as antimetabolites. The design of a second generation of NAs (with a different mechanism of action than antimetabolites) that could be combined with GEM is an utmost objective of our research program. These new drugs hold the promise of improving treatment of PDAC to the benefit of Canadian patients. Over the past few years, we have successfully synthesized new chemical entities that represent an unprecedented class of NAs. These proprietary molecular platforms (patent-protected) display a common feature: the presence of a quaternary carbon center (QUAT platform). NAs from this series have either a *South* or a *North* conformational bias. Interestingly, in the course of this research program we discovered two other molecular platforms. The Pro-QUAT platform bears various prodrugs add-ons addressing typical resistance determinants such as phosphorylation, deamination or cellular uptake. The Met-QUAT platform molecules were designed to interfere with cancer cell metabolism and related bio-energetics processes. When tested *in vitro* for their activity against several human cancer cell lines, two molecules in each Pro-QUAT and Met-QUATplatforms showed significant antiproliferative activity. In pancreatic cell lines in particular (Capan-2 and BxPC3) they showed activity close to GEM (EC_{50} < 10 µM). Various *in vitro* and *in vivo* experiments are planned to further assess the preclinical potential of our lead series (See Specific aims). As suggested by our preliminary results, these novel compounds hold great promise in the context of the very deadly pancreas cancer.

Specific Aims:

Our medicinal chemistry-based research program, proposed herein, is based on the following premises:

- Medicinal chemistry: to synthesize 5-20g of the two lead series (Pro-QUAT and Met-QUAT).
- Biological evaluations (*in vitro*): to determine isobolograms and potential synergy between GEM and the two lead series in pancreatic cell lines (Capan-2, BxPC3).
- Biological evaluations (*in vivo*): to demonstrate improved *in vivo* efficacy of a combination with GEM in xenograft models with nude mice, as compared to GEM alone (BxPC3, Capan2). Their respective *in vivo* efficacy will be determined as a single agent as well.
- Biological evaluations (*in vitro*): to determine potency of the two lead series in GEM-resistant cell lines (PANC-1 and MIA PaCa-2) using GEM as a control.
- Biological evaluations (*in vitro*): to determine selectivity index using MRC-5 non-transformed human fibroblasts.
- Biological evaluations (*in vitro*): Various experiments will be performed in order to give insights into each molecule mechanism of action. HPLC-MS will be used to determine active metabolites for the Pro-QUAT lead series.

List of Key Publications:

1. Prévost, M.; St-Jean, O.; Guindon, Y. "Synthesis of 1′,2′-cis-Nucleoside Analogues: Evidence of Stereoelectronic Control for S_N2 Reactions at the Anomeric Center of Furanosides". J. Am. Chem. Soc. 2010, 132, 12433.

2. Chapdelaine, D.; Cardinal-David, B.; Prévost, M.; Gagnon, M.; Tamburlin, I.; Guindon, Y. "A Stereoselective Approach to Nucleosides and 4̍-thioanalogues from Acyclic Precursors". J. Am. Chem. Soc. 2009, 131, 17242.

3. Duplessis, M.; Cardinal-David, B.; Waltz, M.-E.; Guindon, Y. ; "Stereoselective Quaternary Center Construction via Atom-Transfer Radical Cyclization using Silicon Tethers on Acyclic Precursors". Org. Lett. 2009, 11, 3148-3151.

4. Yvan Guindon, Nucleoside and Nucleotide Analogues with all Carbon Quaternary Stereogenic Center and Methods of Use. Priority date 17 January 2007, publication date 17 June 2008, PCT: US60/881,043".

5. Mochirian, P.; Godin, F.; Katsoulis, I.; Fontaine, I.; Brazeau, J.-F.; Guindon, Y. "A Bidirectional Approach to the Synthesis of Polypropionates: Synthesis of the C1-C13 Fragment of Zincophorin and related isomers" J. Org. Chem. 2011, 76, 7654.

6. Synthesis of Tertiary and Quaternary Stereogenic Centers: A Diastereoselective Tandem Reaction Sequence Combining Mukaiyama and Free Radical-Based Allylation; Benoit Cardinal-David, Brigitte Guérin, and Yvan Guindon* J. Org. Chem. 2005, 70, 776-784.

Investigation of the pathogenesis of ASXL1 mutation in acute myeloid leukemia

NSC-TFRI International Collaborative Research (2013-2016)

Investigators: Keith Humphries, Aly Karsan, BC Cancer Agency; Hwei-Fang Tien, Yuan-Yeh Kuo, Wen-Chien Chou, National Taiwan University College of Medicine

Scientific Summary: ASXL1is the human homolog of Drosophila additional sex combs (Asx), which encodes a chromatin-binding protein required for normal determination of segment identity in the developing embryo. ASXL1was found to be mutated in AML and other myeloid malignancies. A team of researchers at the National Taiwan University have analyzed the clinical implications of this mutation in a large cohort of their *de novo* AML. They found several features of this mutation, including not correlating with a normal karyotype, frequent association with older age, male sex, isolated trisomy 8, RUNX1 mutation, and expression of HLA-DR and CD34, but mutual exclusion with t(15;17), complex cytogenetics, FLT3-ITD, NPM1 mutations, WT1 mutations, and expression of CD33 and CD15. Several studies have shown that ASXL1 mutation is a poor prognostic factor. However, the pathophysiology underlying the mutation remains largely unknown. In this collaborative project, the Taiwan and Canadian researchers propose to explore the mechanisms of ASXL1 that would be of great value in understanding the processes of leukemogenesis and in searching for novel therapy.

Specific Aims:

- To investigate the nature of human ASXL1 mutation *in vivo*. Is ASXL1 mutation a loss-of-function, gain-of-function, or dominant-negative mutation in human AML?
- To understand how the ASXL1 mutation affect epigenetic regulation *in vivo*.
- To answer if ASXL1 mutation alone is sufficient for leukemogenesis.
- To explore the co-operation between mutations of ASXL1 and other genes such as RUNX1.
- To search for any novel therapy.
- Canadian investigators Drs. Humphries and Karsan will work closely with the Taiwan collaborators to analyze the miRNA/mRNA expression profiles, characterize the ASXL1 mutant “knock-in” mouse model, validate the findings in mutations from the discovery cohort, and provide expertise, training, and re-agents for retroviral/lentiviral gene transfer (shRNA and mRNA) to identify collaborating genes with mutant ASXL1 that would accelerate leukemogenesis or suppress leukemogenesis.

List of Key Publications:

1. Chou WC, Huang HH, Hou HA, Chen CY, Tang JL, Yao M, Tsay W, Ko BS, Wu SJ, Huang SY, Hsu SC, Chen YC, Huang YN, Chang YC, Lee FY, Liu MC, Liu CW, Tseng MH, Huang CF, Tien HF. (2010) Distinct clinical and biological features of de novo acute myeloid leukemia with additional sex comb-like 1 (ASXL1) mutations. *Blood* 116: 4086-4094.

2. Kuchenbauer F, Mah SM, Heuser M, McPherson A, Rüschmann J, Rouhi A, Berg T, Bullinger L, Argiropoulos B, Morin RD, Lai D, Starczynowski DT, Karsan A, Eaves CJ, Watahiki A, Wang Y, Aparicio SA, Ganser A, Krauter J, Döhner H, Döhner K, Marra MA, Camargo FD, Palmqvist L, Buske C, Humphries RK. (2011) Comprehensive analysis of mammalian miRNA* species and their role in myeloid cells. *Blood* 118: 3350-8.

3. Starczynowski DT, Morin R, McPherson A, Lam J, Chari R, Wegrzyn J, Kuchenbauer F, Hirst M, Tohyama K, Humphries RK, Lam WL, Marra M, Karsan A. (2011) Genome-wide identification of human microRNAs located in leukemia-associated genomic alterations. *Blood* 117: 595-607.

4. Starczynowski DT, Kuchenbauer F, Wegrzyn J, Rouhi A, Petriv O, Hansen CL, Humphries RK, Karsan A.(2011) MicroRNA-146a disrupts hematopoietic differentiation and survival. *Exp Hematol* 39: 167-178.

5. Hou HA, Lin CC, Chou WC, Liu CY, Chen CY, Tang JL, Lai YJ, Tseng MH, Huang CF, Chiang YC, Lee FY, Kuo YY, Lee MC, Liu MC, Liu CW, Lin LI, Yao M, Huang SY, Ko BS, Hsu SC, Wu SJ, Tsay W, Chen YC, Tien HF. (2013) Integration of cytogenetic and molecular alterations in risk stratification of 318 patients with de novo non-M3 acute myeloid leukemia. *Leukemia* (Epub ahead of print)

Pan-Canadian Early Lung Cancer Detection Study

Terry Fox Research Institute Translational Cancer Research Project (2008-2015)

Co-directors: Stephen Lam, BC Cancer Agency; Ming Tsao, PMCC, UHN

Investigators:

BCCA-VCH: Annette McWilliams, John Mayo, Richard Finley, John Yee, Ken Evans, Paola Nasute

University of Calgary: Alain Tremblay, Paul Burrowes, Paul MacEachern

PMCC, UHN: Heidi Roberts, Geoff Liu, Frances Shepherd, Kam Soghrati, Kazurhiro Yasufuku, John Thenganat, Charlie Chan, Natasha Leighl

Juravinski Cancer Centre: John Goffin, Serge Puksa, Lori Stewart, Allan McLellan, Bill Evans

Ottawa Hospital Regional Cancer Centre: Garth Nicholas, Glen Goss, Jean M. Seely, Kayvan Amjadi

University of Laval: Simon Martel, Francis Laberge, Michel Gingras, Christian Couture

Dalhousie University: Michael Johnson, Daria Manos

Memorial University: Rick Bhatia

Lung Cancer Risk Modeling: Martin Tammemagi, Brock University; Don Sin, UBC; Geoff Liu;

Health Economics & QOL: Stuart Peacock and Sonya Cressman, BC Cancer Agency; Bill Evans, Martin Tammemagi, Natasha Leighl

Blood Biomarkers: Geoffrey Liu, Don Sin

COLD Network (Lung Function): Wan Tan, UBC

Quality Assurance: Nestor Müller, UBC (Radiology); Tom Sutedja, Vrje Universiteit Medical Centre (Bronchoscopy); Adi Gazdar, University of Texas Southwestern Medical Center (Pathology)

Scientific Advisory Committee: Christine Berg, National Cancer Institute, NIH,DHHS; John Field, University of Liverpool Cancer Research Centre; James Jett, National Jewish Health

Funding Partners: The Canadian Partnership Against Cancer, Lung Cancer Canada, Princess Margaret Cancer Centre Foundation, BC Cancer Foundation

Scientific Summary: Sophisticated but relatively expensive technologies such as low-dose, spiral-computed tomography (CT) and autofluorescence bronchoscopy (AFB) exist for detection of early lung cancer. The inclusion of low-cost risk modeling and biomarkers to select population cohorts with the highest risk of lung cancer development may provide a cost-effective application of relatively expensive, yet effective, detection methods. Our objective is to develop a new, multi-modal, early-detection strategy that integrates risk modeling, spirometry, AFB and blood biomarkers with CT for early detection of lung cancer.

- The study has a 4.8% cancer detection rate. Eighty-six percent of the cancers were detected from abnormalities observed at the baseline scan and 14% were incidence cancers.
- CT-scan data from the study was used as a development data set to determine the malignancy of lung nodules on first-screen CT. The study has produced a highly predictive tool based on patient and nodule characteristics to accurately estimate the probability that lung nodules detected on baseline screening CT are malignant. The results were published in the *N Engl J Med* 2013;369:910-919
- The study aims to complete a third round of screening on enrolled participants to contribute additional information regarding the frequency and duration of LDCT screening.

List of Key Publications:

1. Probability of Cancer in Pulmonary Nodules Detected on First Screening CT. Annette McWilliams, Martin C. Tammemagi, John Mayo, et.al. *New England Journal of Medicine* 2013;369:910-919 September 2013.

2. Pro-Surfactant Protein B as a Biomarker for Lung Cancer Prediction. Don D. Sin, Martin Tammemagi, Stephen Lam, Matt J. Barnett, Xiaobo Duan, Anthony Tam, Heidi Auman, Ziding Feng, Gary Goodman, Samir Hanash, Ayumu Taguchi for the Pan-Canadian Early Lung Cancer Study group. Accepted for publication in *Journal of Clinical Oncology*.

Translational research in lung cancer: From molecular markers/targets to therapeutic applications

NSC-TFRI International Collaborative Research (2013-2016)

Investigators: Stephen Lam, Wan Lam, BC Cancer Agency; Pan-Chyr Yang, Chong-Jen Yu, National Taiwan University Medical College and Hospital

Scientific Summary: Lung cancer is the leading cause of cancer mortality worldwide as well as in Taiwan. Delayed diagnosis, early metastasis, poor treatment outcome and rapid emergence of drug resistance are the present obstacles for the management of lung cancer patients. Dr. Martin Tammemagi has developed a lung cancer risk prediction model in never smokers using the PLCO dataset (from the NCI sponsored Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial) (PMID: 21606442). Drs. Tammemagi and Stephen Lam demonstrated that pulmonary function and sputum DNA image cytometry added value to their lung cancer risk prediction model (PMID: 21411501). Dr. Lam's team validated this prediction model in a pan-Canadian lung cancer biomarker screening trial, which was supported by TFRI (PMID: 24004118). The utility of this prediction tool can be tested in never and ever smoker populations in Taiwan. Furthermore, the incremental value of the inclusion of specific genetic biomarkers can be investigated. We hypothesize that *the pan-Canadian risk model is applicable for predicting lung cancer risk in the Taiwan population*. The goal is to validate our PLCO model (validated in a TFRI sponsored pan-Canadian trial) in a Chinese population and determine if genetic markers add incremental value to risk assessment in never and ever Chinese and Caucasian population.

Specific Aims:

- Evaluate the utility of the Tammemagi PLCO model risk model in the Taiwanese population.
- Optimize such a model for early detection of lung cancer in Taiwan.
- Test the incremental value of genetic markers to predict lung cancer risk versus the Tammemagi PLCO model that includes family history and lung function (FEV1%).

Impact: Early detection is critical to the reduction of lung cancer mortality. The development of a risk model optimized for the Taiwan population will have the potential for improving clinical practice. Furthermore, such a risk model can be adapted for other populations in Asia and worldwide.

Three-dimensional nuclear telomeric profiles at the transition from myelodysplastic syndromes to acute myeloid leukemia

Terry Fox Research Institute Translational Cancer Research Project (2012-2013)

Investigators: Dr Macoura Gadji and Dr Sabine Mai University of Manitoba, Manitoba Institute of Cell Biology (MICB)/CancerCare Manitoba (CCMB)/ Genomic Center for Cancer Research and Diagnosis (GCCRD)

Scientific Summary: Despite several studies demonstrating the role of telomere dysfunction in the occurrence of hematopoietic malignancies, little is known about their role in the evolution of myelodysplastic syndromes (MDS) to acute myeloid leukaemia (AML). Our preliminary results show distinct telomeric profiles specific to patients with MDS or AML, and suggest for the first time a chronological and evolutionary process of telomere dysfunction in this disease[1].

General Objective: The main goal is to define the molecular and cellular mechanisms underlying the transformation/progression of MDS to AML and then to identify implicated genes in this process as potential target genes to improve the dire prognosis of MDS and AML.

Specific Aims: To validate the clinical significance of 3D telomere profiling of MDS and AML patients, we will:

- Study patients progressing from MDS to AML over time. Such a longitudinal study will allow for precise 3D telomeric profiling during disease progression.
- Study disease progression from MDS to AML using a unique mouse model (C57BL/6- Tg(V ava1-NUP98/HOXD13)G2Apla/J) and determine the 3D telomere profiles of each mouse and sex and age-matched control at every month or at transformation to AML.
- Define 3D telomeric, cytogenetic and molecular criteria of disease progression from MDS to AML.

Findings: We find a trend of chronological telomere dysfunction in mice[2,3] that is similar to the human condition (see paper 1). Additionally, the two-telomere pathways (two chronological telomere dysfunctions) of progression from MDS to AML previously defined in human samples appear to be confirmed by the mouse samples.

Significance: This project will allow us to validate the 3D telomere profile as a biomarker of MDS and AML and to gain comprehension of the molecular and cellular mechanisms leading to the transition of MDS to AML. We will gain a better understanding of the nuclear processes leading to this transition between MDS and AML that will provide a new basis for new molecular therapeutic strategies aimed at improving the dire prognosis of MDS and AML. This will, in the future, aid in individualized (personalized) patient management**.**

Key List of Publications:

1. Gadji M, Adebayo Awe J, Rodrigues P, Kumar R, Houston DS, Klewes L, Dièye TN, Rego EM, Passetto RF, de Oliveira FM, Mai S., Profiling three-dimensional nuclear telomeric architecture of myelodysplastic syndromes and acute myeloid leukemia defines patient subgroups. *Clin Cancer Res*. 2012;18:3293-3304.

2. Gadji M, Mai S, "Towards validation of three-dimensional nuclear telomeric architecture as a biomarker of myelodysplastic syndromes and acute myeloid leukemias", Oral Presentation : TFRI Prairie Node Symposium, 2013-06-25, Canada, Saskatchewan, Saskatoon.

3. Gadji M, Mai S, "Three-Dimensional Nuclear Telomeric Profiles at the transition of Myelodysplastic Syndromes to Acute Myeloid Leukemias in a mouse model", Poster Presentation : 4th Annual Scientific Meeting of The Terry Fox Research Institute (TFRI), 2013-05-09, Canada, Ontario, Ottawa

A pan-Canadian platform for the development of biomarker-driven subtype specific management of ovarian carcinoma

Terry Fox Research Institute Translational Cancer Research Project (2010-2015)

Investigators: Anne-Marie Mes-Masson, Diane Provencher, Kurosh Rahimi, CHUM Research Centre; David Huntsman, BC Cancer Agency; Anna Tinker, Dianne Miller, Blake Gilks, Brad Nelson, Peter Watson, BC Cancer Agency, VGH; Tony Magliocco, Martin Koebel, Helen Steed; Alberta Health Services; Hal Hirte, Hamilton Health Sciences Centre; Ted Brown, Joan Murphy, Barry Rosen, Helen Mackay, Marcus Bernadini, Patricia Shaw, Blaise Clarke, Amit Oza, UHN; Trevor Shepherd, London Health Sciences Centre; Janet Dancey, Jeremy Squire, Queen's University; Barbara Vanderhyden, Johanne Weberpals, Micheal Fungkee Fung, Ottawa Regional Cancer Center; Walter Gotlieb, Jewish General Hospital, Montreal; Mark Natchigal, University of Manitoba; Patricia Tonin, McGill University; Alain Piché, University of Sherbrooke; Isabelle Bairati; Dimcho Bachvarov, Marie Plante, Bernard Tetu, CHUQ, Université Laval; Robin Uqhart, Dalhousie University; Eva Grunfeld, OICR

Scientific Summary: Ovarian cancer is the second most common gynecological cancer and the leading cause of death from gynecological malignancies. Early detection of ovarian cancer is rare and little is known about the natural history of disease progression. More recently, the notion that ovarian cancer is a single disease has given way to a more sophisticated concept of subsets of ovarian disease that may be associated with very different molecular events. While platinum/taxane-based treatment is currently the gold standard in first-line therapy, failure of this treatment in a significant portion of patients remains a serious problem. Identifying non-responders, and offering these individual alternative first-line treatments, remains one of the most important aspects in the initial clinical management of the ovarian cancer patient, and the focus of future clinical trials.

In order to address these issues, the research team proposes the following specific aims:

- A validated classification system for ovarian cancer that stratifies cases into groups with different natural histories and chemotherapy response rates, and a trained pathology community ready to effectively perform this classification, and a QA program to maintain excellence.
- A cohort of over 2,000 ovarian cancer sub-typed cases (COEUR) to be interrogated for biomarkers predictive of treatment response, and analysis would be extended to address response in sub-type specific ovarian disease.
- The biomarkers with the highest predictive value will be carried forward for correlative studies in sub-type specific ovarian cancer trials.
- A pan-Canadian team of clinical researchers working within a collaborative framework to reduce ovarian cancer mortality.

List of Key Publications:

1. Le Page, C. et al.: Report on a quality control exercise performed on specimens from Canadian biobanks participating in the COEUR specimen repository. *Biopreservation and Biobanking* (2013) 11(2), 83:93.
2. Le Page, C. et al: Predictive & Prognostic Protein Biomarkers in Epithelial Ovarian Cancer –Recommendations for Future Studies: *Cancer* (2010) 2, 913-954.
3. Köbel M. et al.: The biological and clinical value of p53 expression in pelvic high-grade serous carcinomas. *J Pathol.* (2010) 222(2):191-8.
4. Madore J. et al.: Characterization of the molecular differences between ovarian endometrioid carcinoma and ovarian serous carcinoma. *J Pathol.* 2010, 220(3):392-400.
5. Wiegand KC. : ARID1A mutations in endometriosis-associated ovarian carcinomas. *N Engl J Med*. (2010) 14; 363(16):1532-43
6. Barrès V et al. : An essential role for Ran GTPase in epithelial ovarian cancer cell survival. *Mol Cancer*. (2010) 13;9:272

Selective Therapies Program collaboration: Therapeutic targets validation in ovarian cancer

Terry Fox Research Institute Translational Cancer Research Project (2012-2015)

Investigators: Anne-Marie Mes-Masson, Institut du cancer de Montréal, CRCHUM, Université de Montréal; Robert Rottapel, OICR, UofT; Diane Provencher, University of Montreal; Laudine Communal, Institut du cancer de Montréal, CRCHUM, Montreal; Mauricio Medrano, OICR, Fabrice Sircoulomb, OICR

Scientific Summary: Ovarian cancer is the most lethal of gynecological cancer and effective therapies are still lacking as the majority of patients develop resistance to first-line chemotherapy. The therapeutic targets validation project is included in the Selective Therapy Program (STP) developed at OICR and launched by the Terry Fox Research Institute. OICR has identified candidate-essential genes in ovarian cancer cell lines and stratified those expressed at the surface membrane or included in amplified regions. The identification was achieved using an integrative approach combining shRNA-based functional screen, surface protein expression, copy number alterations and RNA-seq based transcriptome[1]. Moreover, the Toronto Recombinant Antibody Center (TRAC) associated with the STP was able to generate therapeutic antibodies targeting the candidates. The challenge is to prioritize potential targets and to estimate the therapeutic relevance of TRAC antibodies. A systematic candidate characterization includes expression evaluation by Western blot (WB) using our 21 Epithelial Ovarian Cancer (EOC) cell lines from different subtypes[2,3]. The preliminary screening by WB confirms the expression of first-selected candidates in most ovarian cancer cell lines. Candidate analysis is subsequently coupled with an immunofluorescence (IF) study on large-scale ovarian cancer tissue micro arrays to correlate protein expression with patient clinical parameters and determine the most pertinent candidates[4]. IF conditions were defined to reveal four colours labeling that allows the simultaneous visualization of epithelium, the nuclear compartment, and can monitor two additional candidates. Accurate analysis of IF results is ongoing using the VisioMorph software. Candidates showing high and frequent expression will be prioritized for a therapeutic antibody evaluation.

List of Key Publications:

1. Marcotte R, Brown KR, Suarez F, et al: Essential gene profiles in breast, pancreatic, and ovarian cancer cells. *Cancer Discov* 2:172-89, 2012

2. Ouellet V, Zietarska M, Portelance L, et al: Characterization of three new serous epithelial ovarian cancer cell lines. *BMC Cancer* 8:152, 2008

3. Letourneau IJ, Quinn MC, Wang LL, et al: Derivation and characterization of matched cell lines from primary and recurrent serous ovarian cancer. *BMC Cancer* 12:379, 2012

4. Ouellet V, Guyot MC, Le Page C, et al: Tissue array analysis of expression microarray candidates identifies markers associated with Tumour grade and outcome in serous epithelial ovarian cancer. *Int J Cancer* 119:599-607, 2006

Efficacy of optically guided surgery in the management of early-stage oral cancer: The Canadian optically guided approach for oral lesions surgical (COOLS) trial

Terry Fox Research Institute Translational Cancer Research Project (2010-2015)

Investigators: Catherine Poh, Scott Durham and Stuart Peacock, UBC; Miriam Rosin, Kitty Corbett, SFU; Calum MacAulay, BC Cancer Agency; Joseph Dort, University of Calgary; Hadi Seikaly, University of Alberta; Paul Kerr, Health Sciences Center, Winnipeg, Manitoba; Karen Kost, McGill University Hospital; Kevin Higgins, Sunnybrook Odette Cancer Center; John Yoo, London Health Sciences Center; Mike Odell, University of Ottawa; Robert Hart, Dalhousie University

Scientific Summary: The COOLS trial is a multicentre Phase III randomized control trial that is evaluating the clinical efficacy of an optical tool to reduce local recurrence of oral cancers and severe dysplasia. The tool identifies alteration of tissue autofluorescence (FV) around oral lesions and uses such change to delineate surgical margins. The study will recruit a total of 400 patients with oral severe dysplasia or higher. Patients will be randomized into either FV-guided (experimental arm) or white-light (current standard of care) surgery.
The trial has 4 goals.

- To collect clinical evidence of the comparative effectiveness of the two treatments.
- To collect molecular and phenotypic evidence in margins to test if FV produces a shift in surgical field, sparing normal tissue while catching high-risk occult tissue.
- To collect relative cost-effective evidence of the two treatments in both the cost per avoided recurrence and the cost per quality-adjusted life years (QALYs) gained.
- To develop a knowledge translation (KT) strategy that will foster the dissemination of FV-guided surgery across Canada and globally.

As of July 31, 2013, we have seven sites are actively recruiting patients at a steady pace: Vancouver, Calgary, Edmonton, Winnipeg, Toronto, London and Halifax. A total of 301 patients have consented and been treated, including 179 with cancer and 122 with high-grade lesions. The patient visit follow-up rate, defined as percentage of anticipated visits that occurred at each specified follow-up time interval, is at an average of 90%.

Continuation of sample pipeline and molecular analysis for margin samples: We have developed a pipeline for sample flow from acquisition, to documentation, histological review, and sample selection for analysis, processing and delivery to laboratories for molecular and phenotypic analysis. Preliminary quantitative tissue pathology (QTP) and loss of heterozygosity (LOH) analysis shows positive correlations of these endpoints with margin evaluation using histology and FV status. Early data suggests that we may be able to spare low-risk tissue at surgical margins.

Health economics team:
The health economics team (Goal 3) has been developing its analysis plan using interim data from the COOLS trial. Unit costs for time and equipment have been applied to surgical capture forms and sub-population analyses have been applied to investigate differences in clinical populations. These methods, and some interim findings, have been submitted and presented at national and international conferences, with feedback from health economics experts. A qualitative “face to face interview” component has been appended to this goal, and has received provisional approval from the UBC Research Ethics Board. Interviews will begin in fall 2013. Interim data analysis will continue as the trial increases its recruitment and follow-up.

Knowledge translation:
Knowledge translation (KT) in the COOLS trial entails health service data collection to prepare for dissemination and scale-up of fluorescence visualization (FV)-guided surgery beyond the trial, if warranted by study results. KT discovery and application throughout the trial is informed by social marketing and diffusion of innovations change theories. The discovery phase has been completed and involved data collection from study site surgeons, pathologists, clinic staff, and patients that explored factors and processes of FV-guided surgery important for clinical practice change.

Future application-phase activities will include developing and testing of an acceptable, appropriate KT scale-up strategy. Both phases use a between-case, compare-and-contrast approach concurrent with qualitative data collection to identify emerging themes and inform subsequent steps.

List of Key Publications:

1. MacLellan S, Lawson J, Baik J, Guillaud M, Poh CF, Garnis C. Differential Expression of miRNAs in the Serum of Patients with High-risk Oral Lesions. *Cancer Medicine*, 2012, 1(2):268–274

2. Saini R., Poh CF. Topical photodynamic therapy: A review and its prospective role in the management of oral potentially malignant disorders. *Oral Diseases*, 2012, doi: 10.1111/odi.12003

3. Lin Jun R., Lubpairee T., Liu KY, Anderson DW, Durham S, Poh CF. Cyclin D1 Overexpression is Associated with Poor Prognosis in Oropharyngeal Cancer. *Canadian Otolaryngology Surgery Journal* 2013, 42(1):23

4. MacAulay C, Poh CF, Guillaud M, Williams PM, Laronde DM., Zhang L, Rosin, MP. High Throughput Image Cytometry Platform for Detection of Suspicious Lesions in the Oral Cavity. *Journal of Biomedical Optics,* 2012; 17(8), 086004.

5. Zhang L, Poh CF, Williams M, Laronde DM, Berean K, Gardner PJ, Jiang H, Lang W, Lee JJ, Rosin MP. Loss of Heterozygosity (LOH) Profile - Validated Risk Predictors for Progression to Oral Cancer. *Cancer Prevention Research* 2012, 5(9):1081-9

6. Macdonald D, Gu Y, Zhang L, Poh CF. Can Clinical and Radiological Features Predict Recurrence in Solitary Keratocystoic Odontogenic Tumours? *Oral Surgery, Oral medicine, Oral Pathology, Oral Radiology*, 2013, 115(2): 263-271

7. Hallani SE, Poh CF, Follen M, MacAulay, C, Guillaud M, Lane P. Ex Vivo Confocal Imaging with Contrast Agents for the Detection of Oral Potentially Malignant Lesions. *Oral Oncology*, 2013, 49(6): 582-90

8. Towle, R, Truong D, Hogg K, Robinson, WP, Poh CF, Garnis C. Global analysis of DNA methylation changes during progression of oral tumourigenesis. *Oral Oncology*. (Accepted Aug 19, 2013)

TFRI-OICR Selective Therapies Program

Terry Fox Research Institute Translational Cancer Research Project (*2008-2009–Seed Fund: 2009-2013–Full Program)*

Investigators: Rob Rottapel, Brad Wouters, Ben Neel, Aaron Schimmer, OCI; David Andrews, Sunnybrook Research Institute; Peter Dirks, Hospital for Sick Children; Daniel Durocher, Samuel Lunenfeld Research Institute, Mount Sinai Hospital; Jason Moffat, Sachdev Sidhu, Lilliana Attisano, UofT; Frank Sicheri, Jeff Wrana, Samuel Lunenfeld Research Institute, Mount Sinai Hospital; David Stojdl, Children's Hospital of Eastern Ontario

Scientific Summary: The Selective Therapies Program (STP) is jointly funded by the Terry Fox Research Institute (TFRI) and the Ontario Institute for Cancer Research (OICR). The STP is a translational program whose objective is to identify novel cancer targets against which new anti-cancer therapeutics can be developed with heightened selective properties. The program has been designed to accelerate the process of target discovery, validation and development by creating a fully integrated team including clinical oncologists, pathologists, cancer cell biologists, molecular biologists, bioinformaticians and medicinal chemists. The team has developed high-throughput screening assays using RNA interference (RNAi) technologies and has performed high-content chemical screens to identify gene targets and first-generation small molecules that specifically sensitize tumours to death or involution compared to normal tissue cell types. The TFRI-OICR Selective Therapies Program is an ambitious translational initiative that addresses the mandate of both the TFRI and OICR to discover and develop new anti-cancer targets with the potential of improving clinical outcomes for cancer patients. Target identification has focused on three tumour types, namely breast cancer, ovarian cancer and pancreatic cancer. To date, 285 genome-wide shRNA screens have been completed in 211 tumour-derived cell lines. Some of these screens have been performed under a variety of physiologic conditions (+/- hypoxia) or as sensitizing screens in response to therapeutic stress (targeted agents or radiation). Essential gene maps and network pathways have been created. Currently, data from functional screens is being integrated with genomic data (expression (RNAseq) and copy number (SNP)) derived from our own cell lines and from available public databases (TCGA) towards the identification of high value targets. Targets are being prioritized for biological validation. The STP is working closely with the OICR medicinal chemistry team and Dev Sidhu (Donnelly Center for Cellular and Biomolecular Research) to develop small-molecule inhibitors and biologics, respectively against validated cancer targets.
The STP (Ontario Node) continues to foster collaboration with other TFRI-funded investigators. For example, a large research collaboration was recently approved by TFRI between investigators in Alberta and Ontario for therapeutic target identification in glioblastoma.

List of Key Publications:

1. Owen, S.C., Patel, N., Logie, J., Pan, G., Persson, H., Moffat, J., Sidhu, S. S. & Sciochet, M.S. (2013) Targeting HER2+ breast cancer cells: Lysosomal accumulation of anti-HER2 antibodies is influenced by antibody binding site and conjugation to polymeric nanoparticles. *J Control Release*. [epub Jul 20].

2. Ma, X., Barthelemy, P.A., Rouge, L., Wiesmann, C. & Sidhu, S. S. (2013) Design of Synthetic Autonomous V_H Domain Libraries and Structural Analysis of a V_H Domain Bound to Vascular Endothelial Growth Factor. *J Mol Biol.* 425, 2247-59.

3. Findlay, G.M., Smith, M.J., Lanner, F., Hsiung, M.S., Gish, G.D., Petsalaki, E., Cockburn, K., Kaneko, T., Huang, H, Bagshaw, R.D., Ketela, T., Tucholska, M., Taylor, L., Botwell, D.D., Moffat, J., Ikura, M., Li, S.S., Sidhu, S. S., Rossant, J. & Pawson, T. (2013) Interaction domains of Sos1/Grb2 are finely tuned for cooperative control of embryonic stem cell fate. *Cell.* 152, 1008-20.

4. Ernst, A., Avvakumov, G., Tong, J., Fan, Y., Zhao, Y., Alberts, P., Persaud, A., Walker, J.R., Neculai, A.M., Neculai, D., Vorobyov, A., Garg, P., Beatty, L., Chan, P.K., Juang, Y.C., Landry, M.C., Yeh, C., Zeqiraj, E., Karamboulas, K., Allali-Hassani, A., Vedadi, M., Tyers, M., Moffat, J., Sicheri, F., Pelletier, L., Durocher, D., Raught, B., Rotin, D., Yang, J., Moran, M.F., Dhe-Paganon S. & Sidhu, S. S. (2013) A Strategy for Modulation of Enzymes in the Ubiquitin System. *Science* 339, 590-5.

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9. Scaling up the systematic hunt for mammalian genetic interactions.Hart T, Moffat J.*Nat Methods*. 2013 May;10(5):397-9. doi: 10.1038/nmeth.2449. No abstract available.

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12. A versatile lentiviral expression system to identify mammalian protein-protein interactions.Mak AB, Moffat J.*Methods*. 2012 Aug;57(4):409-16. doi: 10.1016/j.ymeth.2012.06.005. Epub 2012 Jun 17. Review.

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The Canadian Prostate Cancer Biomarker Network (CPCBN)

Terry Fox Research Institute Translational Cancer Research Project (2010-2016)

Investigators: Fred Saad, Anne-Marie Mes-Masson, Mathieu Latour, Jean-Baptiste Lattouf, Louis-Mathieu Stevens, CHUM; Marie-Paule Jammal, Cité de la Santé de Laval; Jean-Benoît Paradis, Complexe Hospitalier de la Sagamie; Armen Aprikian, Simone Chevalier, Simon Tanguay, Mario Chevrette, Jacques Lapointe, Fadi Brimo, McGill University Health Center; Louis Lacombe, Alain Bergeron Yves Fradet, Hélène Larue, CHUQ; Jeremy Squire, Queen's University; Neil Fleshner, Rob Bristow, Antonio Finelli, Shabbir Alibhai, PMCC; Laurence Klotz, Margaret Fitch, Sunnybrook Hospital; Darell Drachenberg, Manitoba Prostate Center; Martin Gleave, Ladan Fazli, Alan So, Colin Collins, VPC; Simon Sutcliffe, TFRI

Scientific Summary: Prostate cancer is the most commonly diagnosed cancer with an estimated 23,600 new cases in 2013 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 favoured the detection of early stage, and, in some, cases low-grade (Gleason 6 or less) 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 is assembling a large cohort of 1,500 radical prostatectomy specimens, 250 biopsy specimens from intermediate-risk patients treated by radiotherapy and 250 biopsy specimens from low-risk patients who underwent AS. All samples are to microarrayed and associated with extensive clinico-pathological data. This important resource will be useful to validate biomarkers related to prostate cancer patient prognosis to define a nomogram combining the usual clinico-pathological criteria with biomarkers expression. This resource represents a rich validation cohort that will be available to all researchers interested in testing promising biomarkers. Access will be through application to the CPCBN and a positive evaluation by the CPCBN study committee. The CPCBN is focused on the identification of biomarkers that predict risk in order to inform clinical management decisions. Despite the fact that there are clear advantages from a health/quality of life/ health economic viewpoint to AS, its uptake within the Canadian context has not been 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 are poorly documented. Using a database interrogation approach in four different provinces (Quebec, Ontario, Manitoba, British Columbia) the CPCBN is monitoring AS in men that underwent a biopsy in 2010 to provide evidence for the extent of active surveillance uptake in Canada. In depth analysis will be performed to understand the root of any significant differences that might exist between provinces/centres. In parallel, using a focus-group approach, patients and health care providers of the same four provinces are being interrogated to identify perceived barriers and facilitators to AS. Going forward, the rich data collected during the focus-group approach will be used to inform questionnaires that can provide a quantitative measure of the importance of different barriers and facilitators to AS from the patient and practitioner point of view. 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 (active surveillance) from a small group whose disease, despite being apparently low-risk, will progress and result in premature death if left untreated.

Specifics Aims:

Biomarker Core

- Assembly of tissue micro-array- (TMA) based validation platforms:
    - 1,500 radical prostatectomy specimens
    - 250 biopsy specimens from intermediate risk patients treated by radiotherapy
    - 250 biopsy specimens from low-risk patients followed by active surveillance
- Validation of biomarkers:
    - Specific to low-risk disease that will not progress (biopsy based) to safely follow them by active surveillance and avoid therapeutic complication
    - Specific to patients with a high-risk of progression/recurrence to combine their initial treatment with adjuvant therapies.
- Establishment of a nomogram to facilitate prostate cancer patient management.

Knowledge to Action Core

- Snapshot of active surveillance uptake in Canada for the year 2010.
- Identification through focus groups of the barriers in the offer, acceptance and adherence to active surveillance in Canada.
- Organization of a large knowledge-transfer meeting regrouping all TFRI translational projects tackle how to transfer scientific discovery to clinical practice as a means of improving health outcome.

List of Key Publications:

1. Lessard L, Karakiewicz PI, Bellon-Gagnon P, Alam-Fahmy M, Ismail HA, Mes-Masson AM, Saad F. Nuclear localization of nuclear factor-kappaB p65 in primary prostate tumours is highly predictive of pelvic lymph node metastases. *Clin Cancer Res*: 12(19):5741-5 (2006).

2. Koumakpayi IH, Le Page C, Mes-Masson AM, Saad F. Hierarchical clustering of immunohistochemical analysis of the activated ErbB/PI3K/Akt/NF-kappaB signalling pathway and prognostic significance in prostate cancer. *Br J Cancer*: Mar102(7):1163-73 (2010).

3. Klotz L, Zhang L, Lam A, Nam R, Mamedov A, Loblaw A. Clinical results of long-term follow-up of a large, active surveillance cohort with localized prostate cancer. *J Clin Oncol*: 28(1):126-31 (2010).

4. Fleshner NE, Kapusta L, Donnelly B, Tanguay S, Chin J, Hersey K, Farley A, Jansz K, Siemens DR, Trpkov K, Lacombe L, Gleave M, Tu D, Parulekar WR.Progression from high-grade prostatic intraepithelial neoplasia to cancer: a randomized trial of combination vitamin-E, soy, and selenium. *J Clin Oncol*: Jun 10;29(17):2386-90 (2011).

5. Trudel D, Zafarana G, Sykes J, Have CL, Bristow RG, van der Kwast T. 4FISH-IF, a four-color dual-gene FISH combined with p63 immunofluorescence to evaluate NKX3.1 and MYC status in prostate cancer. *J Histochem Cytochem*. 2013 Jul;61(7):500-9.

Medulloblastoma Advanced Genomics International Consortium(MAGIC): Stratifying and targeting pediatric medulloblastoma through genomics

Terry Fox Research Institute Translational Cancer Research Project (2011-2014)

Investigators: Michael Taylor, David Malkin, Hospital for Sick Children; Marco Marra, MSGSC, BC Cancer Agency

Scientific Summary: Brain tumours are the most common solid malignancies observed in children, and the most common cause of pediatric cancer death. The most frequently diagnosed pediatric brain cancer is medulloblastoma. Survival rates have increased to >70% as a result of the surgery, whole brain and spinal cord radiation, and aggressive chemotherapy used in modern treatment. However, survivors are often left devastated with cognitive, physical, reproductive, social, and neurological deficits due to both the disease and its therapy. As survivors are children, they represent a long-term economic and societal challenge to the Canadian health care system, and a significant burden of suffering to Canadian families. Our project is developing tools to identify low-risk patients with medulloblastoma for whom excessive therapies could be reduced in order to improve quality of life, while maintaining current cure rates. While originally thought to encompass one disease, we have demonstrated that medulloblastoma is, in fact, a heterogeneous group of diseases with distinct demographics, clinical presentations, histologies, transcriptomes, cancer genetics, and clinical outcomes. Some children are undoubtedly over-treated and suffer needless complications, while others live for only a very short time despite therapy. To address this heterogeneity, we formed the Medulloblastoma Advanced Genomics International Consortium (MAGIC), which is composed of >50 leading pediatric neuro-oncology centers from around the world who have contributed >1,200 high quality frozen medulloblastoma samples, and a sub-type of paired normal tissues to our tumour bank at SickKids.

Our diverse group of investigators from across Canada and around the world are working towards:

- Discovering biologically driven and clinically important homogeneous sub-groups of medulloblastoma through whole transcriptome profiling of 1,000 tumours, and developing reliable and robust biomarkers for sub-group identification in clinical trials.
- Prioritizing genomic studies on tumours from children with molecularly defined high-risk subgroups of medulloblastoma, and/or a worse quality of life in order to improve functional outcomes and diminish health care expenditures (GE^3LS).
- Discovering tumour sub-group specific somatic mutations in order to inform current clinical trials of targeted therapies and identifying novel genes and pathways already targeted in other diseases, which could be rapidly transitioned to Phase II trials in medulloblastoma.
- Applying economic methodology with Canadian families and oncologists to determine the proper balance between increased risk of tumour recurrence versus improved quality-of-life-after-therapy de-escalation to guide development of the next generation of clinical trials for children with medulloblastoma (GE^3LS).

Through comprehensive, biologically based characterization of medulloblastoma patients we will reduce excessive and futile therapies, identify children at risk for disabling and expensive complications, and identify novel targets for therapy of children who currently have no significant options for clinical trials, and hence little hope. Identification of biomarkers for children who are currently over-treated, in combination with data that will quantify and validate parents' acceptance of therapy de-escalation, will guide the next generation of clinical trials across North America and Europe within the time frame of the project period, thereby providing early societal, economic, clinical, and academic benefits to Canada.

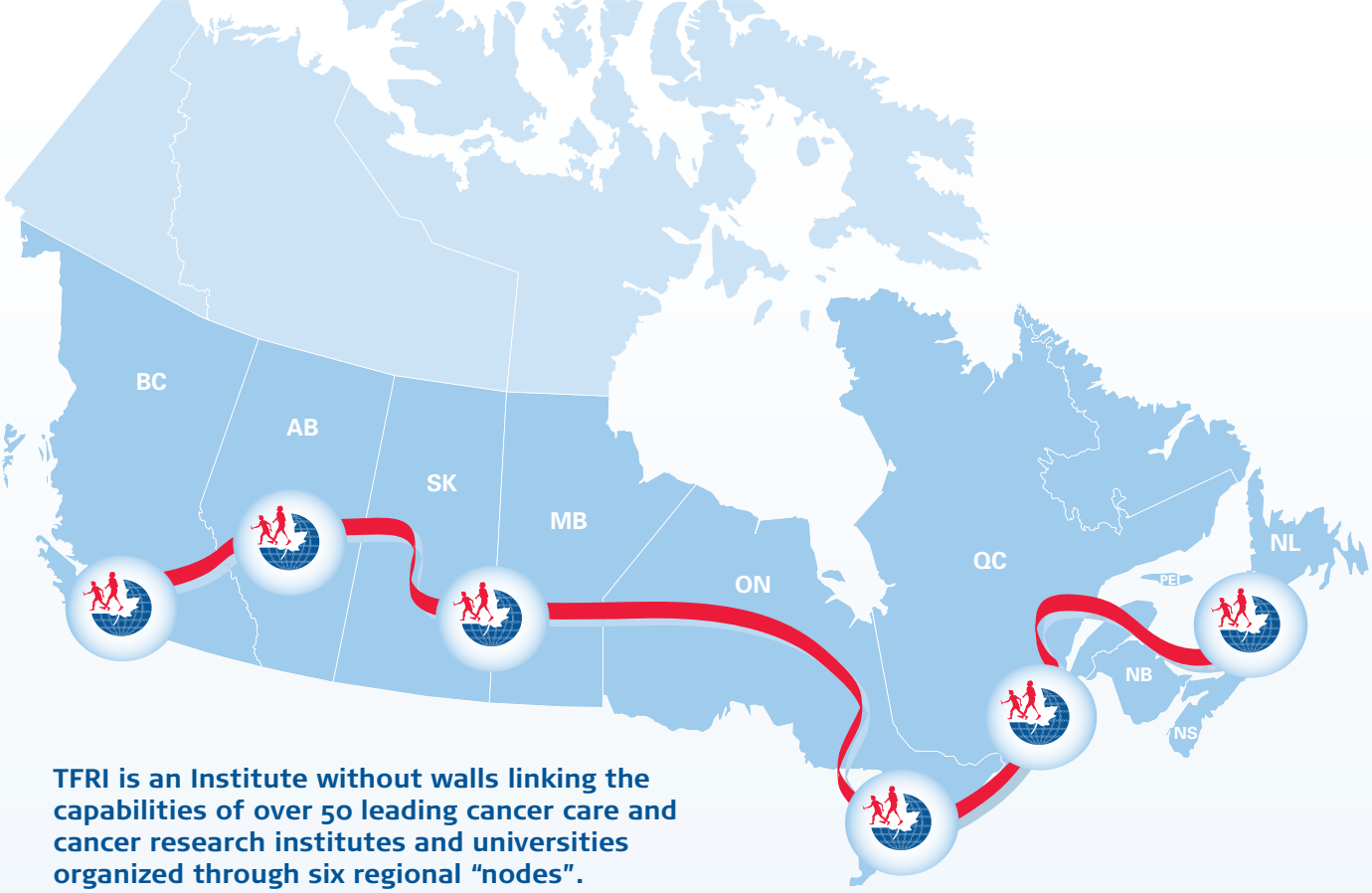
List of Key Publications:

1. Northcott PA, Shih DJH, Remke M, Cho YJ, Kool M, Hawkins C, Eberhart CG, Dubuc A, Guettouche T, Cardentey Y, Bouffet E, Pomeroy SL, Malkin DM, Rutka JT, Korshunov A, Pfister S, Taylor MD. Rapid, reliable, and reproducible molecular sub-grouping of clinical medulloblastoma samples. *Acta Neuropathologica*. 2012. 123(4), 615-626.

2. Northcott PA, Shih DJ, Peacock J, Garzia L, Morrissy AS, Zichner T, Stütz AM, Korshunov A, Reimand J, Schumacher SE, Beroukhim R, Ellison DW, Marshall CR, Lionel AC, Mack S, Dubuc A, Yao Y, Ramaswamy V, Luu B, Rolider A, Cavalli FM, Wang X, Remke M, Wu X, Chiu RY, Chu A, Chuah E, Corbett RD, Hoad GR, Jackman SD, Li Y, Lo A, Mungall KL, Nip KM, Qian JQ, Raymond AG, Thiessen NT, Varhol RJ, Birol I, Moore RA, Mungall AJ, Holt R, Kawauchi D, Roussel MF, Kool M, Jones DT, Witt H, Fernandez-L A, Kenney AM, Wechsler-Reya RJ, Dirks P, Aviv T, Grajkowska WA, Perek-Polnik M, Haberler CC, Delattre O, Reynaud SS, Doz FF, Pernet-Fattet SS, Cho BK, Kim SK, Wang KC, Scheurlen W, Eberhart CG, Fèvre-Montange M, Jouvet A, Pollack IF, Fan X, Muraszko KM, Gillespie GY, Di Rocco C, Massimi L, Michiels EM, Kloosterhof NK, French PJ, Kros JM, Olson JM, Ellenbogen RG, Zitterbart K, Kren L, Thompson RC, Cooper MK, Lach B, McLendon RE, Bigner DD, Fontebasso A, Albrecht S, Jabado N, Lindsey JC, Bailey S, Gupta N, Weiss WA, Bognár L, Klekner A, Van Meter TE, Kumabe T, Tominaga T, Elbabaa SK, Leonard JR, Rubin JB, Liau LM, Van Meir EG, Fouladi M, Nakamura H, Cinalli G, Garami M, Hauser P, Saad AG, Iolascon A, Jung S, Carlotti CG, Vibhakar R, Ra YS, Robinson S, Zollo M, Faria CC, Chan JA, Levy ML, Sorensen PH, Meyerson M, Pomeroy SL, Cho YJ, Bader GD, Tabori U, Hawkins CE, Bouffet E, Scherer SW, Rutka JT, Malkin D, Clifford SC, Jones SJ, Korbel JO, Pfister SM, Marra MA, Taylor MD. Subgroup-specific structural variation across 1,000 medulloblastoma genomes. *Nature*. 2012. 488, 49-56.

glossary

BCCA	BC Cancer Agency
BCCRC	BC Cancer Research Centre
CDRD	Centre for Drug Research and Development
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
MSGSC	Michael Smith Genome Sciences Centre
OCI	Ontario Cancer Institute
OHRI	Ottawa Hospital Research Institute
OICR	Ontario Institute of Cancer Research
PMCC	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
UofT	University of Toronto
VGH	Vancouver General Hospital
VPC	Vancouver Prostate Centre



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