September 2019 (IMP-20) Competition Awarded Impact Grants

Listed in alphabetical order

Bhatia, Mick
McMaster University

Targeting responsive microenvironment to prevent relapse of human acute myeloid leukemia

Acute Myeloid Leukemia (AML) is a cancer of the myeloid blood lineage characterized by rapid accumulation of non-functional immature myeloid cells (blasts) in the bone marrow (BM) ultimately leading to anemia, leukocytosis, leukocyte dysfunction and hematopoietic system failure. Research over the past 4 decades has led to the widespread clinical use of Cytarabine (or **Ara**binofuranosyl **C**ytidine [AraC]) as a front-line treatment that achieves excellent remission rates of 50-85% in adult AML patients. Unfortunately, relapse occurs in most patients within 2 years amounting to 5-year survival rates of less than 20%.These clinical results strongly suggest that alternative treatment strategies and approaches are required prolong remission, including a better molecular and cellular understanding of the reason for relapse in response to chemotherapy. Quiescent AML stem cells (LSCs) have been believed to be responsible for reinitiating AML disease after chemotherapy remission induction. Results from our previously funded CCS Impact award provided key insights into the remission and AML regeneration process during chemotherapy, and revealed LSCs are in fact targeted by chemotherapy, consistent with two additional independent reports published in parallel. In our study, we identified a transient subset of cells responsible for regeneration of AML hierarchy that amounts to relapsed disease (***Cancer Cell, 2018***), we termed, Leukemic Regenerating Cell (LRC). We demonstrate that chemotherapy itself effects the local and broader cellular microenvironment (***Nature Cell Biology, 2017***) for sustaining AML disease and LRCs. These studies suggest that new non-cell autonomous approaches in conjunction with chemotherapy may be essential to assure better outcomes for AML patients. **Our renewal proposes to continue the translational steps of these initial findings to define niche targeting drugs to reduce relapse and improve BM transplantation.**

Human LSCs are operationally defined by xenograft transplantation of AML patient-derived cells into immune-deficient mouse strains and represent a powerful tool that several groups have used, including ours (***CELL 2012***) to discover drugs and understand AML cells vs. those that may affect microenvironment (***JEM 2014, and Nature Cell Biology 2017)***. Our goal is to generate pre-clinical data necessary to support Phase I trials that will target LRCs through combined drug and cellular modulation of the BM niche. Our strategy includes the following fundamental goals and specific objectives:

Objective 1. Identifying transient AML Regenerating Factors Produced From Chemotherapy. Improve standard of care in AML by defining and blocking transient factors produced in response to chemotherapy that cause activation of LRC regeneration *in vivo*.

Objective 2. Activating BM Adipogenesis to Inhibit AML and Enhance Healthy HSC Regeneration. Develop pre-clinical approaches for AML niche targeting by characterizing the *in vivo* effects of drug induced BM adipogenesis on AML disease regeneration and define responsive cellular subsets that comprise the BM niche by in situ BM single cell transcriptomics.

Objective 3. Kinetic Characterization of AML Emergence in Response Chemotherapy and Niche Modulation. Resolve and identify subsets of AML residual cells post cytoreductive chemotherapy in combination with niche remodeling drugs using sequential single cell transcriptome analysis.

The outcome of our studies examining microenvironment modulation in AML will provide cellular and molecular targets to test new combinations of consolidation therapies during and after remission induction, as well as drug adjuvants to HSC transplantation of AML patients to prevent relapse.

With continued support from CCS, our assembled team of scientists and physicians will be able to continue to form the foundation for first-in-person trials for novel management of AML. To our knowledge, **no other group is studying these specific approaches to combat AML relapse in conjunction with standard of care chemotherapy**. Given poor survival rates of this disease due to relapse prevalence post remission induction, it is time to examine innovative approaches.

Dick, John
Princess Margaret Cancer Centre - UHN

Cellular and molecular mechanisms underlying AML initiation and evolution

Our **Vision** is to understand the earliest steps of when and how acute myeloid leukemia (AML) develops and to use this knowledge to innovate methods for early AML detection and therapeutic intervention focussed on targeting the preleukemic phase.
Rationale: AML has a dismal prognosis, particularly in older patients. With current CCSRI funding we traced the origins of AML back to preleukemic hematopoietic stem cells (preL-HSC) that acquire initiating leukemogenic mutations. We also discovered that the origins of relapse lie buried within rare leukemia stem cells (LSC) already present at diagnosis. These findings highlight the difficulty in devising new therapies to target AML either at diagnosis or relapse: extensive Darwinian branching evolution before diagnosis endows robustness to the leukemic ecosystem leading to early development of relapse-fated cells; mutational evolution initiates in HSC and continues in LSC where stemness processes (self-renewal, dormancy, prosurvival pathways) render these clonal propagating cells difficult to target. While much work in the field is aimed at utilizing new genomic and pathway information of AML cells to identify vulnerabilities for targeting, our proposal takes a different direction. Our overarching premise is that curing patients with AML will remain exceedingly difficult. We are focussed on understanding the earliest stages of leukemic evolution with the ultimate goal of targeting preleukemia before it progresses to AML. We propose to identify individuals within large population cohorts and with blood disorders who are fated to develop AML within the next decade. Such capabilities open a long window of opportunity to target preL-HSC, restraining their capacity to clonally expand thereby preventing full blown AML from developing.
Our **Research Plan** takes advantage of three main findings we made with prior funding: 1) individuals in the general population who are fated to progress to AML can be identified up to a decade earlier; 2) we have expertise in isolating human HSC and undertaking single cell molecular and functional analysis; and 3) we have generated key multi-omic datasets on normal HSC subsets and begun to define core determinants of stemness providing essential comparator information to uncover how preleukemic processes hijack normal HSC to create preL-HSC. Here, we are using two cohorts to study early leukemia development. **Aim 1:** We will use biobanked enrollment samples from the Canadian Partnership for Tomorrow Project (CPTP), a prospective longitudinal cohort of healthy individuals, some of whom eventually developed AML. **Aim 2:** We will use serial samples banked over 5-10 years from patients diagnosed with MPN who progressed to AML. For both aims, AML mutations will be identified using our new, high precision smMIP target sequencing on the preleukemic samples. We will perform high resolution single cell (sc) index sorting for HSC and progenitors (HSPC) followed by scRNA-seq and scATAC-seq and parallel genotyping determine whether the cell carries the AML mutations or not. **Aim 3:** From the collected expression and epigenetics datasets, we will use a battery of computational approaches to develop regulatory networks that are predicted to drive stemness for each paired (wildtype vs mutant) HSPC. Candidates predicted to cause preL-HSC to self-renew and clonally expand will be validated in functional in vitro and in vivo assays that evaluate human HSC expansion; with a subset chosen as candidates for future translational studies aimed at preventing preL-HSC from progressing to AML.
Expected Outcomes: We expect to identify the regulatory networks that define the stemness states of preL-HSC and wildtype HSC, thereby providing an opportunity to determine how the ARCH-AML mutations hijack the stemness programs of HSC. The key regulators of these regulatory networks represent therapeutic targets that our functional studies will validate. We expect to move several of these on to translational therapeutic studies in xenografts aimed at preventing preL-HSC from clonally expanding. Overall, targeting pre-leukemia is the foundation for a radically new prevention strategy for AML.

Dirks, Peter
The Hospital for Sick Children

Reimagining glioblastoma as a consequence of aberrant neural tissue repair

Glioblastoma (GBM), the most common primary brain malignancy, has an appalling 5-year survival of less than 10%1, making it an essentially incurable cancer. Our group has shown that GBM has its growth driven by tumour subpopulations (glioblastoma stem cells, GSCs) that sit at the apex of a developmental hierarchy that persists in the neoplastic context. In analysing a multi-omics database of primary GBM samples and corresponding tumour-derived GSCs with data from over 115 patients, we have discovered a new paradigm for thinking about GBM that is leading to a wealth of new hypotheses of novel therapies and biology that we propose to study. This data strongly suggest that wound healing responses and neural regenerative programs are an essential feature of GBMs, particularly at the level of the GSCs. In this proposal, we will re-think the origins, biology and treatment strategies of GBM based on these exciting findings.

Our RNA-seq data of the GSC lines indicates that there are two major types of GSCs. One class (proneural) resembles the developing brain, including neuronal differentiation, and expresses pathways involved in neural development, signaling and morphogenesis. The second class (immuno-mesenchymal) expresses a large number of inflammatory pathways and epithelial to mesenchymal transition and extracellular matrix pathways. The proneural class has a gene expression profile closest to neural stem cells, young astrocytes, or oligodendrocyte precursor cells and the immuno-mesenchymal most closely resembles reactive astrocytes, and a newly discovered astrocyte subtype critical for neural tissue wound response.

Hypothesis:

Two dominant GSC states underlie every adult human glioblastoma. The two GSC states reflect dual responses of mutant neural precursor cells to injury and/or inflammation. Treatment approaches must simultaneously consider the unique biology of these states. The presence of these states also suggests that GBM may in part originate as a consequence of abnormal injury/inflammatory responses of genetically mutant but proliferation-permissive cells in the adult CNS.

Experimental Plan:

We propose two broad aims to test our hypotheses:

Aim 1: We will use multi-omics computational drug and mechanism prediction methods to identify drugs and novel drug combinations designed to simultaneously target all cell populations present in GBM, and we will evaluate these using in vitro and in vivo models.

We will predict a range of drugs and novel combination therapies targeting GSCs and their progeny, using our extensive multi-omics GSC database. We will use diverse experimental models, ranging from cell cultures to mouse xenografts, combined with single cell genomic read outs, to identify the most translationally promising therapeutic drugs and drug combinations predicted computationally.

Aim 2: Determine the role of injury or inflammation in GBM genesis and phenotype.

Using mouse models of GBM, we will determine how induction of three types of brain injury (inflammation/sepsis, wounding or trauma, and low dose radiation) affect normal cellular responses in the presence or absence of GBM associated mutations, therefore probing the earliest mechanisms of GBM initiation, and studying the role of injury/inflammation in GBM initiation and tumour phenotype.

Summary:

We aim to define a new paradigm in GBM biology, with wound healing and regeneration as an important component, with knowledge interchange between human and mouse models. This opens multiple avenues to discover novel therapy candidates that target GBM heterogeneity as a whole, and will open up new thinking regarding mechanisms of GBM initiation and potentially, with better understanding of the root causes of GBM, even prevention strategies. Ultimately, we hope that our work will lead to new GBM therapy clinical trials and medium- to long-term advancements in GBM clinical care resulting in improved outcome for this terrible disease.

Hieter, Philip
University of British Columbia

Setting a trap for cancer cells: high throughput screening for protein trapping synthetic lethal targets for cancer therapy

Personalized, genotype-driven, anti-cancer therapies are emerging as a new paradigm for tumour treatment. These approaches choose therapeutics based on the constellation of mutations and/or expression profiles in a patient's tumour. Key to this approach is development of genotype-linked therapeutic agents. The concept of synthetic lethality provides an opportunity to develop new precise anti-cancer therapeutics. Synthetic lethality (SL) occurs when disruption of two gene products individually is non-lethal but simultaneous disruption of both gene products results in cell lethality. The synthetic lethal partners of a cancer-specific mutation would be potential anti-cancer drug targets. Synthetic lethal interactions can identify drug targets for non-druggable cancer lesions. Synthetic lethality-based therapeutics would provide selectivity killing of tumour cells, since by definition, healthy cells lacking the tumour-specific lesion will not be affected.

Although synthetic lethality-based therapies hold promise for expanding the range of genotype-targeted therapy, most synthetic lethal interactions that have been identified have not translated to the clinic. We hypothesize that the success rate of SL-based therapeutics can be improved by analyzing the properties of the few SL interactions that have been developed into effective therapeutics. The two classes of SL-based anticancer therapeutics that have made it to the clinic are PARP and topoisomerase inhibitors. The most effective PARP and topoisomerase inhibitors trap their protein target on the DNA resulting in a toxic protein-DNA complex that requires a functional DNA damage response for repair, which leads to differential killing of tumour cells with dysfunctional DNA damage responses.

Current synthetic lethal therapeutic target screening approaches, while effective in identifying some synthetic lethal genetic interactions, do not accurately model synthetic lethal interactions between cancer mutations and chemical inhibition of synthetic lethal partners. High throughput screening paradigms based on CRISPR-Cas9 or RNAi can not

discover targets that require the presence of the target protein such as the case of protein trapping. New approaches are needed to discover potential therapeutic targets that can be trapped or inhibited to generate toxic protein-DNA, protein-RNA, or protein-protein complexes. We have developed a yeast-based system that exploits point mutations that more accurately mimic the activity of chemical inhibitors to screen for cancer relevant SL interactions. We have conducted a small proof-of-principle study characterizing the effect of trapping the FEN1 nuclease and the Chl1 helicase and have found them effective at eliciting a SL response even in the presence of WT FEN1 or Chl1. We propose to scale-up screening efforts by generating large-scale yeast synthetic genetic arrays containing point mutations in enzymes known to interact with DNA. We will generate arrays with mutations in yeast DNA metabolism genes and arrays that heterologously express mutated human DNA metabolism genes that do not have yeast homologs. Heterologously expressed human genes in yeast can be used to screen for genetic interactions and chemi-genetic interactions with inhibitors.
Therapeutic targets will be analysed using deep mutational profiling to identify protein domains and amino acids that are important for protein trapping and synthetic lethality. A better understanding of the specific amino acids and domains required for the genetic interaction can direct the development of more specific assays to screen for small molecules that can phenocopy the SL effect.
Our proposed research will discover new therapeutic targets for development that would otherwise be missed in current large-scale CRISPR-Cas9-based screens and provide valuable information that can be exploited to better design therapeutic small molecule inhibitors.

Ikura, Mitsuhiko
Princess Margaret Cancer Centre - UHN

Translating discoveries in RAS mechanistic biology into cancer therapeutics

The RAS oncoproteins are frequently mutated at one of three specific amino acids (G12, G13, or Q61) and thus become “hyper-activated” in approximately 30% of all solid tumours, primarily pancreatic, lung, and colon cancer (K-RAS) and melanoma (N-RAS). However, the development of clinically effective RAS-directed cancer therapies has been largely unsuccessful. Thus, RAS mutant cancers remain among the most refractory to available treatments. Because of this clinical need, this “old foe” has become an intensive research target in recent years. One of the major knowledge gaps in RAS biology is a lack of detailed understanding of how RAS proteins work on the membrane, where they must be localized to activate effector proteins and drive signaling. Another issue in the field is an incomplete understanding of how RAS signaling cross-talks with other pathways, and which pathways are critical in establishing specific subgroups of tumours. The answers to these questions must come from protein-based studies, hence we aim to elucidate critical structural and functional signatures of mutant RAS and to develop new therapeutic strategies. Our mechanistic discovery approaches will then be tested using cancer cell lines, patient-driven organoids, and patient-driven xenografts. We have established an active collaborative research team at Princess Margaret and beyond, towards developing novel strategies to treat RAS-driven cancers.
RAS lacks classical drug-binding pockets, thus targeting this challenging protein will require innovative approaches. We have pioneered novel methods to enable structural studies of KRAS and its interactors on the membrane surface, and provided proof-of-principle for targeting the KRAS:membrane interface (*Cell Chem Biol* 2018). In this program, we endeavour to discover and optimize novel molecules that exploit this unique membrane-dependent mechanism of action. RAF kinases are key RAS effector proteins that are activated by RAS in a manner that requires the presence of a membrane. We will also seek to uncover mechanistic details of the activation of RAF by RAS, and investigate the role of KRAS multimerization on the membrane surface. This may reveal interaction interfaces that could be targeted to block RAF activation. We also aim to characterize pathways that ‘cross-talk’ with KRAS to modulate signaling, towards discovery of indirect approaches to inhibit oncogenic KRAS signaling. In particular, we will study tyrosine phosphorylation of RAS by Src kinase, which impairs effector binding, and dephosphorylation by Shp2 phosphatase. A major focus will be given to three K-RAS mutants G12D, G13D, and Q61H, in which we identified potential *Achilles heels* in their structures, which will be targeted.
Regarding methodologies, we have already established a strong foundation using the nanodisc technology with a variety of biophysical and biochemical methods. We will also combine *in silico* screening with *in vitro* binding assays using our chemical libraries for the rational drug design in collaboration with OICR. Inhibitors discovered from those studies will be functionally tested using established cancer cell lines in collaboration with internationally well-known experts. Once validated in cancer cell lines, we will then move to more clinically relevant models, namely patient-derived xenografts (PDX) and organoids (PDO), which are readily available from our PM-CC collaborators. These resources will allow us to explore the efficacy and specificity of our K-RAS inhibition approaches in different cancer types with different K-RAS mutation status. Ultimately, we hope to test our K-RAS inhibitors at a clinical setting, which is readily available to this program through our strong clinical trial program at PM-CC.

Muller, William
McGill University

Targeting EZH2 in breast cancer: role of epigenetic modification in modulating cross talk between tumour epithelial cell and its microenvironment

Altered epigenetic regulation of gene expression through modifications of DNA and chromatin is a critical factor in breast cancer progression. In spite of this, the relationships between oncogenic signaling and epigenetic regulation in breast cancer remain largely unknown. Pharmacological inhibition of activated oncogenes is the basis for rationally designed "targeted therapies" that specifically inhibit the growth and survival of tumor cells. However, while many such drugs are now clinically approved, they are plagued by the inevitable emergence of resistance in the vast majority of patient. Importantly, some studies have suggested the involvement of epigenetic gene regulation in this process. This phenomenon is poorly understood and further investigation of aberrant epigenetic processes in breast cancer is clearly warranted. In this proposal, we will use a combination of Genetically Engineered Mouse Models (GEMMs) and *in vitro* model systems of human breast cancer to evaluate the role that Ezh2-dependent epigenetic re-programming plays in both breast cancer progression and the acquisition of resistance to targeted therapeutics. Finally, we will examine the feasibility of targeting the altered epigenetic control programs of breast cancer cells to enhance the efficacy of established therapeutics and overcome resistance to these agents.

Routy, Bertrand
Centre de recherche du CHUM

Fecal microbial transplantation to increase immunotherapy activity in melanoma and non-small cell lung cancer

BACKGROUND: Despite immune checkpoint blockers (ICB) acting as the standard of care in metastatic melanoma (MM) and metastatic non-small cell lung cancer (mNSCLC), long-lasting control appears in a minority of patients. Immune-related adverse events are also a major hurdle, and therefore innovative methods to improve ICB efficacy and reduce toxicity are urgently needed. Our team demonstrated that a key component of ICB efficacy lies in the gut microbiome. Indeed, studies in germ-free (GF) mice have shown that the antitumor activity of ICB requires certain gut bacteria. Building on these results, we have shown that antibiotic (ATB) use prior to ICB initiation negatively impacts clinical outcomes in MM and mNSCLC patients. Subsequently, gut microbiome profiling revealed that high bacterial diversity and specific commensal (non-pathogenic) bacteria (*Ruminococcaceae*, *Bifidobacterium*in MM and *Akkermansia muciniphila*in NSCLC) were associated with cytotoxic CD8+T and CD4+T cell activity correlated with improved clinical response to anti–PD-1. Favorably shifting the gut microbiome composition, translating into more inflamed T cells in the tumor microenvironment, can provide an opportunity to circumvent ICB primary resistance. Our unpublished results show that microbiome modification with fecal microbiota transplantation (FMT) from healthy donors favorably transformed microbiome composition in preclinical models leading to stronger ICB anti-cancer responses and a protective effect against immune related colitis. In Canada, FMT in capsules are routinely used to treat *C. difficile* infections among other conditions and Western University has a dedicated unit that screens healthy volunteers directed by Dr. Silverman. Based on the aforementioned results, Dr. Maleki (Western) group has received Health Canada approval (NCT03772899) and already safely included 2 patients in the first combination trial of FMT capsules with approved ICB in patients with MM in Canada (phase I).
OBJECTIVE: Our goal is to create the first randomized phase II clinical trial in Canada to combine FMT and ICB to evaluate if microbiome modification through FMT can safely improve the systemic anti-cancer immunity and ICB efficacy in untreated MM and mNSCLC patients. In parallel, FMT in GF mice will be performed to better understand the mechanisms linking the gut microbiome to ICB response.
METHODS: Upon completion of the phase I in March 2019, 146 patients with untreated MM and NSCLC eligible for anti-PD-1 first-line immunotherapy will be randomized 1:1 to receive FMT by capsules plus standard-of-care anti-PD-1 or standard-of-care anti-PD-1 alone. Capsules will be generated from healthy donors selected via a stringent protocol already in placed at Western University. After screening, donors will be selected based on sequencing profiles displaying elevated bacterial diversity and high levels of *A. muciniphila*, *Faecalibacterium prausnitzii*and *Bifidobacterium*. In addition to safety evaluation and assessment of clinical outcomes as per RECIST 1.1 criteria, this study will include microbiome metagenomic profiling in collaboration with Dr. Corbeil (Laval University platform and bioinformatic team). Extensive immunomonitoring as well as tumor immunology will also take place. To narrow the knowledge gap of how the microbiome influences ICB response, FMT from the same patients and donors will be used for experiments in the CRCHUM GF facility where microbiome profiling, metabolomics and immune profiling will be performed.
IMPACT: We hope to establish a strong understanding on how to best modify the gut microbiome of cancer patients to potentiate ICB anti-cancer activity and diminish the risk of immune-related colitis. This clinical trial with strong translational studies will help generate hypotheses on microbiome composition and immune profiling post-FMT and how these changes potentially increase ICB long-term efficacy. Overall, we believe that this study has the ability to

radically change how melanoma and non-small cell lung cancer are treated in Canada.

Wrana, Jeffrey
Mount Sinai Hospital

Revival stem cells in colorectal cancer

Colorectal cancer (CRC) is the 2nd most commonly diagnosed cancer in Canada (Source: Canadian Cancer Society) and arises from the intestinal epithelium, which is highly proliferative and under homeostatic conditions turns over every 1-2 weeks. Homeostatic turnover of the intestinal epithelium is driven by Lgr5+ intestinal stem cells (Lgr5+ ISCs) that are also thought to be the cell of origin for CRC. WNT pathway mutations drive expansion of Lgr5 cells and formation of adenomas, which are precursor lesions to CRC and subsequent mutations in Ras, TGFB signalling and p53 then drive progression. We showed that the Hippo pathway is integrated with both TGFB and WNT signalling and that Hippo was critical in both intestinal regeneration and initiation of adenomas. This work highlighted how chronic inflammatory diseases, such as ulcerative colitis, might drive repeated cycles of epithelial repair and thus promote tumour initiation and progression. We also showed by single cell profiling, that regeneration of the intestine requires a new type of stem cell that we termed the revival stem cell (revSC) that are also found CRC. ***Thus, overall goal of this proposal is to define the importance of regenerative versus homeostatic stem cell dynamics in sporadic and colitis-associated CRC initiation and progression.***
Objectives and Methodology:
To test our hypothesis, we will pursue three aims:
Aim 1) Determine how TGFB induces revSCs.
Our preliminary studies showed that TGFB potently induces revSCs in mouse Apc mutant intestinal spheroids and that inhibition of TGFB signalling suppresses the numbers of basal and TGFB-induced revSCs. Here, we will:
a) Define how TGFB regulates revSC function in vivo by knocking out the TGFB type II receptor either broadly in the intestinal epithelium, or specifically in revSCs or Lgr5+ stem cells and quantify intestinal regeneration and adenoma initiation.
b) Explore the molecular mechanisms underlying TGFB-Smad induction of revSCs by using ChIPSeq to define how TGFB-Smad and Yap converge on the revSC genome to control epigenetic dynamics and gene expression.
Aim 2) Characterize human revSCs.
We discovered Clu+ revSCs in mice and more recently, that Clu+ cells comprise 5% of human adenocarcinomas, but are rare in the normal epithlelium. Thus, in this aim we will:
a) Characterize human intestinal revSCs by single cell profiling of human WT, APC-/-, and CRC organoids to identify conserved and human-specific revSC features and by examining stem cell dynamics in TGFB and 5-FU treated spheroids.
b) Characterize human revSCs in vivo during early tumour initiation by transcriptomic profiling of single cells from human dysplastic IBD lesions and adjacent normal colon obtained during surgical resection.
c) Probe RevSC and LGR5+ stem cell dynamics in human CRC initiation and progression using tissue microarrays by in situ hybridization with stem cell markers and analyze signalling pathway activity by immunohistochemistry. Moreover, we will develop a high dimensional spatial map of revSCs and Lgr5+ ISCs using Imaging Mass Cytometry.
Aim 3) Define how revSCs function in tumour initiation and progression.
Here, we will define how revSC versus Lgr5+ ISC dynamics drive CRC initiation and progression, by employing cell type-specific ablation coupled to lineage tracing to define if revSCs and Lgr5+ stem cells will reconstitute each other after transient ablation, if concomitant ablation will abolish Apc and carcinogen-induced tumours and we will test the requirement for signalling networks ISC dynamics.
Signifcance
Defining previously unknown cell types in intestinal biology that link regenerative signaling to cancer initiation and progression promises to open a new avenue for development of cell type-directed therapies, rather than pathway-specific approaches that dominate the cancer drug development space. Furthermore, we expect that revSC-like populations will soon be identified by single cell profiling in other regenerative tissue, and thus defining how revSCs are regulated and their role in cancer may provide important conceptual advances in cancers beyond CRC.

Wyatt, Alexander
University of British Columbia

Practical genomic stratification to enable tailored treatment of DNA repair defective prostate cancer

Despite recent approval of new treatment options for metastatic castrate-resistant prostate cancer (mCRPC), many patients do not initially respond, and all eventually progress. Multiple trials of targeted therapies have shown a lack of efficacy and even harm in unselected populations. Progress is critically hindered by an inability to enrich trials with

patients that could potentially respond. In mCRPC, a disease with significant molecular and clinical heterogeneity, this is largely due to the difficulty in obtaining relevant tumour tissue for genomic stratification. However, the development of techniques to profile metastatic disease from plasma circulating tumour DNA (ctDNA) offers the prospect of a new generation of clinical trials.

We developed a novel clinical trial platform where mCRPC patients are screened and then treated on the basis of their ctDNA profile. In patients with progressing mCRPC, we have previously demonstrated that ctDNA recapitulates somatic mutations, copy number changes, and structural rearrangements derived from sequencing of matched metastatic tissue. Importantly, in a pilot phase of our clinical trial platform, we have demonstrated feasibility through screening more than 200 Canadian patients in the past 14 months, with 81 enrolled to preliminary treatment cohorts. In this project, we will prospectively profile 340 Canadian mCRPC patients in order to identify DNA damage repair (DDR) pathway defective tumours. DDR is defective in 25% of mCRPC, and these tumours are molecularly distinct and associate with aggressive clinical courses when standard of care approaches are used. However, defects involving members of the DNA homologous recombination repair pathway (e.g. BRCA2 biallelic loss) can be synthetic lethal in tumours in the context of PARP inhibition and can also result in exquisite sensitivity to platinum-based chemotherapies such as carboplatin. Other DDR alterations such as mismatch repair gene loss (e.g. MSH2 mutations and complex rearrangements) and CDK12 kinase domain mutations result in highly immunogenic tumours that are potentially vulnerable to therapies activating the immune response.

Our hypothesis is that mCRPC molecular subtype as determined by ctDNA sequencing is predictive of clinical benefit to therapies that exploit defective DDR. To test this, whole blood from eligible patients at 11 cancer centres across Canada will be shipped to a central laboratory where we will use a validated custom ctDNA analysis approach that identifies hypermutation and mismatch repair gene (MSH2/6, MLH1, PMS2) mutations, deletions, complex rearrangements, CDK12 mutations and associated tandem duplication phenotype, as well as pathogenic biallelic alterations in homologous recombination repair genes (BRCA1/2, ATM, and other FANC or RAD51 family members). In Aim 1, we will evaluate the clinical activity of combination immune checkpoint inhibition in patients with and without evidence of mismatch repair defects. In Aim 2, we will evaluate carboplatin chemotherapy in patients with and without homologous recombination repair pathway defects.

The two proposed clinical trial arms described in this proposal will provide evidence of the feasibility of using ctDNA to select patients with mCRPC who have the greatest potential to benefit from immune checkpoint inhibitors and carboplatin chemotherapy. These therapies have significant toxicity and (in the case of immunotherapy) expense, but low efficacy in unselected mCRPC. Tailoring the use of these treatments will therefore reduce patient morbidity and healthcare costs, as well as improve the length of life for the subset of men who do benefit. Importantly, if the pre-specified level of anti-tumour activity is seen, we will execute definitive randomized phase 2/3 studies within our national cooperative trial group. Overall, we believe that our innovative clinical trial platform is a model for allowing Canadian patients to gain rapid access to potentially active agents, and for advancing a strategy of rational treatment selection for people with cancer.