Precision Cancer Drug Discovery

Introduction

Over the past decade, CECR and CFI investment in the Vancouver Prostate Centre (VPC) has established international excellence in discovery cancer research (e.g. genomics, structural and molecular biology, computer science), integrated with globally-recognized programs in translational research and clinical science. VPC is at the leading edge of global efforts in precision oncology (MyPOP), distinguished by our integrated team-based organization and cores that integrates oncology, genomics, pathology, biobanking, computer science (including algorithm development, bioinformatics, CADD), engineering, biology, *in vivo* models and drug technologies. Our translational cancer research approach uniquely integrates genome sequencing from tissue biopsies and ctDNA assays in plasma to pathway analysis and biologic mechanisms of treatment resistance in unique models systems. Unlike many other centres that focus on genomic evaluation of biopsies at diagnosis, our genomic profiling focuses on treatment resistant and metastatic cancers. Liquid biopsies using ctDNA coupled with longitudinal clinical follow-up (before, during and after treatment) enable study of the evolution of treatment resistance in patients. These key components of precision oncology are established at VPC and already guiding treatment decisions in patients with advanced cancer.

The functional interpretation of genomic data sets is focused on treatment resistant cancer and enabled by deep and broad expertise in cancer biology (metastasis, invasion, drug resistance), structural biology, preclinical modeling (in patient-derived xenografts, organoids), drug discovery, and molecular imaging. These capabilities support functional evaluation of genes, prioritization of cancer-driving targets, and preclinical/clinical pharmacology testing of promising drug candidates. Underpinning this richly diverse and collaborative environment is an established track record in traversing the bench-to-bedside gaps, with programs in experimental therapeutics, Phase I, II and III clinical trials.

PCDD Approach

Advances in genome sequencing are rapidly expanding the repertoire of putative drug targets induced under selective pressures of therapy. While these novel variants and interaction sites are relevant targets for cancer therapeutics, they are a challenge to drug because they are not easily accessible to small molecule inhibitors. PCDD will integrate into VPC the structural biology expertise, including advances in cryo-electron microscopy (cryoEM) at UBC, to enable near atomic resolution structure (~3 Å) of previously intractable proteins and protein complexes to guide structure-function data and drug-protein interactions. The unprecedented molecular details and resolution of these structures now provide an entirely new avenue of structure-guided drug discovery targeting these molecules that VPC can capitalize on.

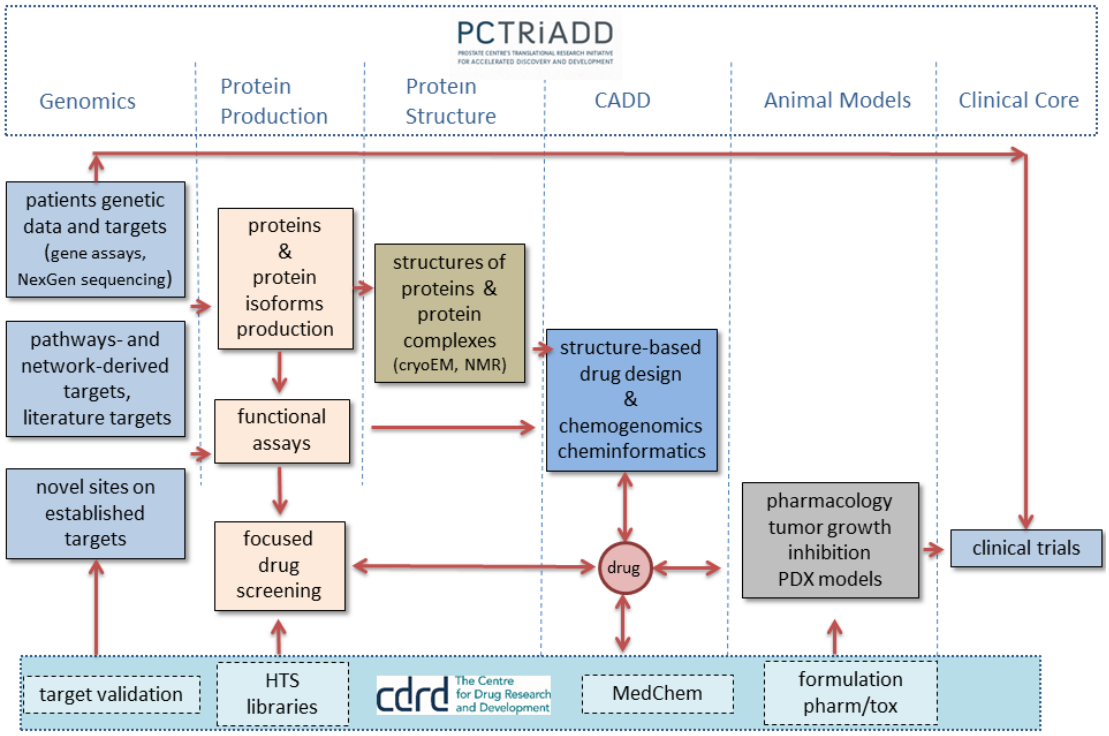
The VPC has been awarded a Canada Excellence Research Chair (CERC) in Precision Cancer Drug Design (PCDD) that will lead a research program that aims, over seven years, to revolutionize the way we discover novel anti-cancer drugs, assisted by use of companion genomic biomarkers in a precision oncology framework. VPC scientists have made world-leading discoveries in cancer genomics and biology, and expanded the repertoire of putative drug targets driving cancer treatment resistance that emerge as a function of clonal evolution. These novel targets are often not amenable to traditional drug screening, and there is an unmet need and unique opportunity for the CERC to rapidly resolve protein structure and link this to computer-assisted discovery of drug inhibitors. Recent innovations at UBC and VPC in structural biology and cheminformatics facilitate characterization of surface-exposed, protein-protein and protein-DNA interaction sites to enable computer-augmented drug design (CADD), a technology fundamental to our CERC recruitment. The CERC will thus integrate VPC's world-leading genomics, structural biology, translational cancer research and CADD expertise to

accelerate discovery of cancer drugs and predictive biomarkers to more precisely treat specific subsets of patients with novel drivers of cancer treatment resistance. Outcomes will include breakthroughs in understanding disease progression; discovery of new drugs to control treatment resistance; and enhanced capacity to translate new drugs in early phase clinical trials across Canada, to collectively nurture growth in Canada's biotechnology sector.

Over the next five years, PCDD will allow VPC to build its drug-discovery program by integrating genomic discovery of novel targets and rapid resolution of protein structure with CADD (**Figure 1** below) to:

1) Expand capacity to identify new cancer targets;
2) Gain new capacity to purify target proteins and resolve structure using cryoEM;
3) Increase processing for *in silico* drug design, cheminformatics, and AI to match drug to new protein target;
4) Increase ability to define pathways for proof of concept and IP;
5) Enhance project management to support commercialization of drugs.

Figure 1: Precision Cancer Drug Design



The VPC, embedded within an academic (UBC) and tertiary healthcare environment (VCH, BCCA), integrates key capabilities necessary to translate discovery of new targets into novel drugs, and include ability to:

1) identify novel targets using serial genome sequencing data in treatment resistant cancers linked to clinical outcomes and serial monitoring of response to therapy using our ctDNA assays to detect mechanisms of resistance and develop companion biomarkers;
2) discover novel drivers and rapidly define biologic mechanisms in unique model systems;

3) rapidly determine candidate protein structures using the recently acquired Titan-Krios cryoEM;
4) perform CADD, including virtual screening, cheminformatic modeling and molecular dynamic simulations for discovery of small molecule drug prototypes;
5) access an extensive collection of cancer models for drug activity and contextual lethality *in vivo*, including patient-derived xenografts (PDXs), organoids, and cell-line based models of drug resistant cancer; and
6) integrate programmatically with clinical trials experts and ctDNA assays as companion diagnostics.

These attributes, collectively managed under one organization, are unique in Canada and rare world-wide, particular when linked to the promise of quantum computing.

BC government support ($10M over 5 years), levered by recent CFI awards to VPC for genomics (C. Collins CFI #33440, $5.7M) and CADD (A. Cherkasov CFI #36194, $23.1M) along with UBC's Structural Biology (CFI # 33611, $5.7M) integrates gene identification to prioritized protein production, high-throughput protein structure acquisition (using cryoEM), with CADD in the VPC Therapeutics Development Core. New genomic and cryoEM instruments enable 3D structure resolution of novel drug targets and conduct drug discovery in the era of precision oncology (with the focus on treatment resistant prostate, bladder, and kidney cancers). These recent CFI awards introduce leading-edge technologies that, when combined with CECR oversight, have enhanced discovery of novel cancer targets and companion diagnostic biomarkers and expanded CADD capabilities in cancer drug discovery, while also enabling further industry partnerships and commercialization.

The key focus of this PCDD program integrates a protein-centric approach to drug discovery, capitalizing on the revolutionary cryoEM and world-class structural biology group (led by Dr. Natalie Strynadka, a Tier 1 CRC and Howard Hughes Medical Institute Senior Research Scholar) at UBC Life Sciences Institute. CADD relies on atomic resolution structures of target proteins typically elucidated by NMR spectroscopy or X-ray crystallography. However, protein structures of many relevant cancer targets are too large, diverse, or have inherent conformational flexibility that make them difficult to purify and study using conventional approaches. CryoEM will help solve this problem by providing a low resolution "molecular mask" in which to fit crystal or NMR structures determined for individual components to generate atomic resolution models of the macro-molecular complex. Recent remarkable advances in cryoEM now deliver structural information on minute quantities of proteins to a resolution of <3.5 Å. This "resolution revolution" has redefined the boundaries of structural biology, providing 3D structural information on target proteins and protein complexes to enable target screening using CADD.

This evolving capacity to acquire protein structure links powerfully to our large-scale computational infrastructure. After the structure of a cancer target is determined, large scale *in silico* high throughput screening of >100 million molecules is conducted to identify a focused group of probable hit substances. The CADD core at VPC, led by Dr. Cherkasov, uses *in silico* high throughput virtual screening of massive electronic collections of purchasable chemicals to identify novel drug leads, which then inform design of focused libraries, thereby accelerating drug discovery and decreasing costs of early drug development. The resulting high-resolution structural information is integrated in well-established cores in VPC to support an iterative cycle of *in vitro* structural and computational studies, combined with focused library design and *in vivo* cell-based assays, to rapidly optimize candidate leads into potential anti-cancer drugs. These new and expanded capabilities in VPC integrate sequencing, structural biology and computer science to galvanize discovery of CADD-guided

cancer therapeutics and accelerate advancement of early IP-protected candidates through value-adding bioassays, pharmacology studies in novel model systems, and onwards to early clinical trials all within VPC.

A key component of the PCDD program was recruitment of a world-leading structural biologist to accelerate resolution of protein structural at the atomic level using cryo-Electron Microscopy (cryo-EM). This opportunity was enabled by the Canada Excellence Research Chair (CERC) competition, for which the VPC was awarded $20M to recruit Dr. Sriram Subramaniam. Dr. Subramaniam, a global expert in cryo-EM, was recruited from the National Institutes of Health in U.S. He will integrate VPC's world-leading genomics, structural biology, translational cancer research and CADD expertise to accelerate discovery of cancer drugs and predictive biomarkers to more precisely treat specific subsets of patients with novel drivers of cancer treatment resistance.

Current Development and Commercialization Portfolio

The VPC has many drug candidates and biomarkers in its portfolio, several moving from preclinical Proof of Concept to formalized clinical development, while others remain at earlier stages of development. Legacy gift funding strategically supports value-added advancements along the development pipeline, achieving milestones from preclinical to Phase I first in human.

Novel AR inhibitors

The androgen receptor (AR) protein consists of 3 domains: the ligand binding domain (LBD) contains the androgen binding site (ABS) and binding function 3 (BF3) pocket; the N-terminal domain (NTD) contains sites for transcriptional activation and interaction with the LBD for dimerization; and the DNA binding domain (DBD) contains the DNA binding (p-box) and dimerization site (d-box);. The AR is activated by steroid recruitment to the ABS resulting in nuclear translocation, AR dimerization via the DBD d-box and engagement of the functional dimeric assembly at target-genes by the two p-boxes. Conventional AR antagonists block steroid binding at the ABS but only temporarily delay AR-driven tumour growth due to emergence of mutations in the LBD or expression of truncated variants (i.e. ARV7) lacking an LBD, signifying a drug-resistant state termed castration resistant prostate cancer (CRPC). Drs. Cherkasov and Rennie used X-ray crystallography and *in silico* screening to identify, validate, and guide med-chem creation of small molecule inhibitors of the AR-BF3 and DBD d-box sites.

i. **AR DBD P-Box Inhibitors**. UBC/VPC licensed the AR DBD p-box inhibitor program to Roche in Q4 2015. Novel cellular and biochemical screening assays now help identify compounds that effectively bind AR variants - specifically ARV7.
ii. **AR BF3 Inhibitor**. All current AR inhibitors target the mutation prone LBD ABS. While men initially respond well, resistance invariably develops through a variety of mechanisms. We developed small-molecule inhibitors targeting the BF3 pocket, important for coregulatory recruitment, AR transactivation, and N- and C-termini interactions. We applied our CADD pipeline to the AR crystal structure with *in silico* screening of 6 million candidates to identify VPC-13556 binding to the BF3 site with inhibition of AR mutation variants, including those conferring resistance to enzalutamide. Med-chem optimization to improve bioavailability and pharmacokinetic properties derived VPC-13789 with

improved IC50 values (comparable to enzalutamide) and *in vivo* tumour growth inhibition. UBC has filed IP will help optimize prodrug design, *in vivo* pharmacology, and toxicology studies with Phase I clinical trials by 2022.

iii. **AR D-Box Inhibitors**. Our CADD core is helping develop small molecules that block the dimerization interface on the DBD—the D-box, an unexplored but attractive target given the need for dimerization for AR activity. Of relevance to treatment resistant disease, our *in silico* and experimental pipeline identified VPC-17005 to inhibit nuclear AR homodimer and AR/ARV7 heterodimer formation, transcriptional activity, and cell growth of AR/ARV7 positive cell-lines. UBC is filing IP and CECR funded two rounds of medicinal chemistry required to identify a lead series. A pre-clinical lead compound(s) will be selected by potency in biochemical and *in vitro* assays, D-box binding specificity, acceptable rodent bioavailability, and efficacy in xenograft models. VPC will conduct first-in-man trials on post abiraterone- or enzalutamide-treated patients with recurrent, but AR-driven, CRPC, selected based on their ctDNA to define the spectrum of AR genomic alterations that correlate with drug-resistance.

Targeting Hsp27

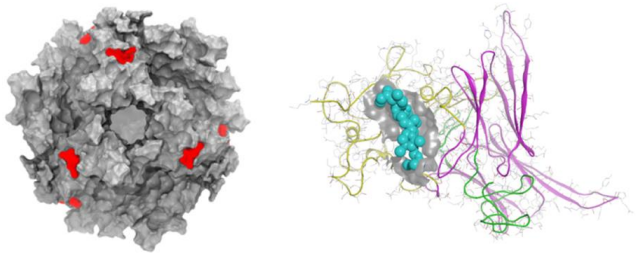


Figure 2: VPC-27 interacts with Hsp27 N-terminal domain. Multi-meric crystal structure of Hsp27 and binding site of VPC-27 on the NTD shown by the space-filling model colored in light blue.

Hsp27 supports oncogenic driver signaling and is situated as a 'Hub" at the center of many pathways regulating response of a cell to therapeutic stress. Dr. Gleave was the first to credentialize Hsp27 as an anti-cancer target, and discovered the antisense drug OGX-427 as the first Hsp27 inhibitor to show anti-cancer activity in prostate and bladder cancer when administered in combination with standard-of-care treatments. While our preclinical and human trials showed, for the first time, single agent activity for an Hsp27 inhibitor (OGX-427) in cancer, a more potent and orally active inhibitor, with higher stability, may improve cancer activity. Indeed, VPC-27 sensitizes prostate and lung cancer to AR and EGFR pathway inhibitors.

A multidisciplinary program in VPC was the first to define the 3D crystal structure of Hsp27. As a first-in-world example of targeting a difficult to drug protein, our CADD core identified a novel drug, VPC-27, that inhibits the NTD of Hsp27. Clinical studies in CRPC and lung cancer are set for 2020.

VAR2 based drug conjugates

VPC scientist Dr. Daugaard developed novel tumour targeting technologies based on recombinant VAR2CSA proteins (rVAR2) from the malaria parasite *Plasmodium falciparum*. rVAR2 proteins bind oncofetal chondroitin sulfate (ofCS) glycosamino-glycans expressed in most human cancers, but restricted to placenta in normal tissues. Daugaard is exploiting this binding system in novel therapeutic and diagnostic strategies. This high-impact paper was published in Cancer Cell.

VPC is developing this technology along 3 therapeutic tracks**:** 1) VAR2 Drug Conjugate (VDC) therapy: The VAR2 technology can be used as a delivery system to specifically position cytotoxic compounds in the tumour environment. The lead drug VDC886 (co-developed with Zymeworks) consists of rVAR2 fused to a highly toxic

hemiasterlin analogue. CGMP manufacturing commenced in 2018-19 with IND application in 2020. 2) Chimeric Antigen Receptor (CAR) T cell immune-therapy: A series of 1st generation CAR T cell products armed with rVAR2 have been developed and lead CAR variants will be selected for further pre-clinical testing**.** CGMP manufacturing of CAR T cell product commenced in 2018. 3) Oncolytic virus therapy: A recombinant rVAR2-expressing oncolytic virus has been developed with Virogin and is in preclinical testing.

SEMA3C inhibitors

Dr. Ong recently discovered SEMA3C as a driver of cancer growth and treatment resistance, activating multiple receptor kinase pathways (EGFR, ErbB2, MET) in a cognate ligand-independent manner via Plexin B1. High SEMA3C expression is associated with CRPC, its over expression promotes cancer cell growth and treatment resistance, and its silencing delays tumour progression. We recently identified Plexin B1 as the primary receptor that mediates SEMA3C signaling in cancer and its knockdown abrogated SEMA3C induced growth. Since SEMA3C is a secreted factor, it is amenable to targeting with protein-based therapeutics, such as Fc-fusion proteins like Enbrel (TNFRII-Fc), aflibercept (VEGFR-Fc), and abatacept (CTLA-4). These fusion proteins are well precedented therapeutics with high regulatory approval rates among all drug classes. We engineered (and patented) a recombinant Plexin B1 extracellular domain decoy receptor fusion protein (PB1SD-Fc) comprised of the sema domain of Plexin B1 fused to the Fc region of immunoglobulin IgG1 to improve its *in vivo* half-life analogous to Enbrel (TNFRII-Fc). PB1SD-Fc inhibits SEMA3C- and androgen-induced cell growth and delays CRPC progression *in vivo*. Studies are ongoing for cell-line optimization, scale up, and cGMP manufacturing of the PB1SD: Fc fusion protein, for IND enabling and planned Phase I human studies.

MCT4 inhibitors

Dr. YZ Wang found that CRPC, like many other cancers, exhibit elevated glycolysis for bioenergetics to increase glucose consumption, which yields lactic acid to promote pro-oncogenic angiogenesis, invasion, and particularly, suppression of local anticancer immunity. Lactic acid secretion is facilitated by MCT4 in highly glycolytic cells and its overexpression is observed in CRPC, while MCT4 inhibition using antisense oligonucleotides (ASOs) inhibits cancer growth, decreases glucose metabolism and lactic acid production, and enhances anticancer immunity. Computer augmented drug design was employed to model MCT4 protein and interactions with small molecule inhibitors to identify leads that inhibit MCT4. A patent for 23 human-specific MCT4 ASO sequences has been filed.

Circulating tumour DNA (ctDNA) Biomarker Assays

The VPC pioneered use of plasma ctDNA for profiling mCRPC. Tumour DNA is constantly released by tumour cells undergoing apoptosis where it mixes with 'normal' cell-free DNA (cfDNA) in blood. The study of ctDNA is complicated by two major factors, firstly that cfDNA is typically highly degraded (fragment size ~150bp), and secondly that it can be very diluted by normal cfDNA (ctDNA fraction/percentage can be as low as 0.01% or >95%). Unlike tissue biopsies (where one can estimate tumour cellularity by pathology prior to sequencing), there is no way to determine the 'ctDNA fraction' before sequencing. Ultimately this means that for the profiling of genomic aberrations in cfDNA from one requires a sensitive, tailored and multi-pronged approach.

VPC helped establish a suite of custom tools and bioinformatic pipelines designed precisely for ctDNA in advanced prostate cancer. We reported in landmark JAMA Oncology and JNCI papers that ctDNA defined genomic alterations better than metastatic tissue biopsy, links AR copy number gain and point mutations to resistance to abiraterone and or enzalutamide, and identifies wide spectrum of genomic alterations in mCRPC, including clinically-relevant changes in DNA repair gene (e.g. BRCA) and PI3K pathway alterations. At ASCO 2017 we presented our Phase II data demonstrating for the first time the prognostic impact of genomic alterations in the context of mCRPC. We are at the leading edge of validating and commercializing this assay, with a $1M grant from PCF. Over the past 3 years we established a unique biobank of plasma cfDNA collected from hundreds of mCRPC patients across Canada, as well as a concerted province-wide liquid biobanking program led by VPC Senior Research Scientist and Chief Medical Officer & Vice President of BC Cancer, Dr. Kim Chi, which represents one of the world's largest collections of CRPC tumour DNA matched to curated clinical follow-up. We developed a specific targeted assay spanning 73 mCRPC driver genes and enabled by PC-TRiADD, now lead Canada's first Phase II umbrella trial prospectively enrolling patients based on their ctDNA profile (with Canadian Clinical Trials Group).

Targeting BRN2 for neuroendocrine prostate cancer (NEPC)

About 25% of CRPC patients develop treatment-induced NEPC (t-NEPC) that have a small cell morphology and express neuroendocrine markers (CHGA, NSE, SYP) instead of adeno-markers like PSA. To define molecular mechanisms that facilitate development of t-NEPC, PC-TRiADD developed unique pre-clinical models to identify key drivers, including SRRM4, PEG10, and BRN2. BRN2 is a neural POU-domain transcription factor that is a central driver of t-NEPC. Since the crystal structure of BRN2 is not resolved, Dr. Zoubeidi built its structure using a homology modeling approach. Molecular dynamic simulations further define protein conformation and an active site was identified that was subjected to *in silico* screening with 4 million small-molecules docked into the DNA binding domain (DBD). 62 chemicals were selected for bench testing and structure activity modelling, with 2 promising compounds further prioritized for stability/efficacy optimization and further preclinical testing. This work led to a recent high impact paper in Cancer Discovery, and a $1.5 M translational acceleration grant from Prostate Cancer Canada (PCC).

Targeting Myc

Myc is a transcription factor that forms a heterodimer with Max protein that is associated with treatment resistance, t-NEPC, and poor outcome. The Myc-Max dimer is at the centre of a vast communication network within cells which binds DNA and broadly regulates expression of many genes involved in cell growth and survival; however it is difficult to drug. Based on the 1.9 Å X-Ray structure of the Myc-Max heterodimer bound to its DNA recognition sequence, we built an *in silico* model of the Myc-Max dimer and identified a novel targetable site where potential inhibitors could bind and disrupt the dimer's transcriptional activity. From this model, we identified and tested 200 potential small molecules binders. The consecutive experimental evaluation of these molecules resulted in identification of 20 hits with micro molar potency (≤ 20 μM) in many PCa, BCa (including a triple negative cell line) and bladder cancer cell lines.

Targeting Gli

The Gli family of transcription factors regulate cell growth, motility, invasion, metastasis, and steroidogenesis. Dr. Buttyan identified a novel pathway activating Gli in PCa cells mediated by the interaction of transcriptionally-active AR with the Gli3 protein at a highly conserved region in its C-terminus referred to as the "Protein Processing Domain" (PPD). ARs compete with β-TrCP and blocks Gli3 proteolysis, maintaining Gli3 in a transcriptionally-active conformation. AR-Gli complexes accumulate at Gli recognition elements on DNA to drive transcription of Gli targets. Dr. Buttyan identified decoy peptides derived from the C-terminus of Gli2 that compete with Gli3 for AR binding and suppress growth of CRPC cells better than enzalutamide. Small molecule inhibitors of Gli are available but these are difficult to administer due to their extreme hydrophobicity and significant side effects. PCDD funding will help identify small molecule inhibitors that interfere with AR binding to Gli proteins and block cancer cell growth. This is an early stage development project with an objective to secure IP. While there are patents for Gli therapeutics in cancer they target the Gli activation domain or Gli binding to DNA. We have purified Gli-AR complexes that will be analyzed via cryoEM to define structure of the interactive protein docking domains. Dr Cherkasov will use these structures to reveal small molecular inhibitors of the Gli3-AR interaction using the CADD core.

IL-33 and Cancer Immune Escape

Recent advances in immunotherapy have significantly improved outcomes for some patients with cancer. However, selecting patients that may respond to emerging immunotherapy modalities remains a clinical challenge. One mechanism that may aid our understanding of patient non-responsiveness to immunotherapy is termed "immune-escape", where genetically unstable tumours undergo the selective pressure of immune-surveillance, yielding tumours that have lost functional antigen processing machinery (APM) components, resulting in reduced expression of functional major histo-compatibility complex (MHC or HLA) molecules. The fidelity of the APM is crucial for tumour recognition by tumour-specific cytolytic T lymphocytes, and APM deficiencies in tumours may render selected tumours "invisible" to adaptive immune responses. By corollary, immune-escape may subvert immunotherapies such as immune checkpoint blockade Inhibitors directed at CTLA-4 and PD-1 that seek to enhance adaptive immune responses. Dr. Wilf Jefferies found that Interleukin 33 (IL- 33) acts as an immune-biomarker where low expression by prostate cancer is associated with a 3.4 year more rapid recurrence after surgery, as compared to IL- 33 high expressing tumours. In addition IL-33 induced the expression of APM in tumour cells, reversing immune-escape, enhancing cancer immune-surveillance and reduced metastases preclinical models.

Based on these clues, Dr. Jeffries hypothesized that since Type 2 innate lymphocytes (ILC2) are developmentally and functionally dependent on IL-33, ILC2s may have a hitherto undescribed role in promoting and mediating anti-cancer immune responses. We now have compelling evidence that adoptive transfer of activated ILC2 cells drastically reduces tumour growth rate, influencing the immune response to tumours, and therefore, may provide a generalized approach to cancer immunotherapy. When compared to anti-cancer T cell therapies, such as chimeric antigen receptor positive (CAR+) T cells, the ratio of adoptively transferred ILC2s to tumour cells is ~ one ILC2 to ~30-40 tumour cells (vs ~5-10 CAR+ T cells to one tumour cell). These discoveries have been patented by members of the VPC and we plan to translate and commercialize these technologies as clinical diagnostic tools and therapeutics.