October 2019 (i2I-20) Competition Awarded Innovation to Impact Grants

Listed in alphabetical order by Panel

I1a Biomarkers and Genomics

Morin, Gregg
BC Cancer, part of the Provincial Health Services Authority

Clinical application of a targeted proteomic classifier for triple negative breast cancer

Problem to be investigated: Triple negative breast cancer (TNBC) is a heterogeneous group of diseases that tends to afflict younger women and is not amenable to targeted therapies with endocrine or anti-HER2 agents. Studies of aberrant events at the RNA and DNA levels have not clearly led to clinical tests that can guide therapeutic choices in these aggressive cancers. During our Innovation phase, we developed new proteomic technologies applicable to archived breast cancer surgical pathology tissues and identified proteomic subtypes of TNBC which may be able to predict which adjuvant systemic treatments will most benefit these patients. **Objectives**: We will build on the existing data and specimens from our Innovation phase, together with materials from international Phase III clinical trials of TNBC, to optimize a targeted proteomics panel suitable for 1) cost effective and rapid application to clinical specimens and 2) reliable identification of the four key subtypes of TNBC evident in the full proteome classification during the Innovation phase. These groups include basal-immune hot, basal-immune cold, mesenchymal, and luminal androgen receptor/HER2-enriched proteomic subtypes. The basal-immune hot group is characterized with adaptive and innate immune response proteins, infiltrating T cells, and significantly improved survival. We will then prove applicability of the targeted assay to large clinical trial series, while testing the hypothesis that the basal-immune hot group will predict patient benefit from the chemotherapy drug capecitabine. **Methodology**: We will employ the novel SP3-Clinical Tissue Proteomics (SP3-CTP) technology, developed in PI Morin's lab and proven to work on standard formalin-fixed paraffin-embedded (FFPE) breast cancer tissues during our CBCF-funded Innovation phase. This technology can quantify 7000-9000 proteins per FFPE sample, and when coupled with our novel isoDoping technology, developed in the Innovation phase study, enables the mass spectrometer to quantify 100s of low abundance biological important proteins that are normally missed. SP3-CTP requires significantly lower input quantities than other proteomics techniques applicable to pathology specimens. In Aim 1, the proteome signatures of the 4 TNBC clusters will be refined by the SP3-CTP/isoDoping analysis using an independent set of TNBC patient FFPE samples. A targeted selected reaction monitoring (SRM) mass spectrometry assay for the signature panel will be developed, optimized for variability, reproducibility, and accuracy, and standardized to classify the 4 clusters. In Aim 2, we will evaluate the SRM classifier assay on FFPE samples from two clinical trials which randomized women to capecitabine vs. conventional chemotherapy, using formal prospective-retrospective study designs capable of generating level 1 evidence for clinical validity. These include FinXX, a study of adjuvant capecitabine from which we have access to 111 TNBC patient FFPE specimens, and CIBOMA, a study of maintenance capecitabine in TNBC from which we have access to 681 patient FFPE specimens. **Significance of the research to cancer**: This research program can contribute on four distinct levels. First, we can demonstrate the efficient, large-scale applicability of SP3-CTP technology to breast cancer specimens in a manner that could be readily adapted to archived clinical specimens from other cancer types. Second, we can validate a proteomic classification of breast cancers which may have distinct advantages over existing RNA and DNA classifiers, particularly at the level of dissecting immune microenvironments and predicting drug responses. Third, we may be able to identify which women with TNBC should receive capecitabine, an effective but toxic chemotherapy regimen. Finally, we will generate the type of evidence required to secure access to specimens from emerging immunotherapy trials of breast cancer, early results from which suggest a subset of women with triple negative breast cancer do benefit from very expensive and sometimes toxic checkpoint inhibitors – a situation where newer and better predictive biomarkers may have special value.

I2 Imaging and Technology Development

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Combined 4FMFES and FDG-PET imaging in advanced breast cancer: a phase II study

Estrogen Receptor (ER) is a crucial prognostic factor and treatment target in breast cancer patients. Knowledge of its status greatly influences the choice of the optimal course of treatment. Pathological evaluations of primary tumor, axillary nodes, and metastases are the only confirmatory approach to ER status determination and are limited to known and accessible sites. However, it is known that many advanced breast cancer patients harbor diseases presenting inter-tumor or temporal ER heterogeneity, as ER expression can vary between tumor foci and can evolve during treatment and at time of recurrence, hence the need for whole-body, non-invasive assessment of ER status. In the last decades, 16α-[18F] fluoroestradiol (FES) was developed and evaluated as an ER-targeting positron emission tomography (PET) tracer. FES correlated with ER expression, and recently was shown to be able to predict hormone therapy response.

Our Center designed and evaluated 4-fluoro-11β-methoxy-16α-[18F] fluoroestradiol (4FMFES), a successor PET tracer for ER imaging. Paired comparison during a phase II clinical trial showed that 4FMFES produced images of better quality, with less overall non-specific signal than FES. It resulted in a significantly improved tumor contrast and tumor detectability using 4FMFES-PET leading to increased diagnosis confidence in early-stage breast cancer compared to FES-PET. Those results demonstrated that, as of now, 4FMFES-PET is the best imaging modality worldwide for whole-body ER status determination, but further validations are necessary to position this method as a standard and essential tool for breast cancer management. Like what was observed for FES-PET, preliminary data suggest that 4FMFES-PET combined with FDG-PET will yield very high sensibility for breast tumor detection, each method being complementary.

In continuity with previous work, we seek to expand our clinical knowledge of this high-potential diagnostic imaging through the following main objective:
Launch a phase II clinical trial to explore the full potential and benefit of 4FMFES-PET in combination with FDG-PET for advanced ER+ breast cancer patients to demonstrate it is an essential tool for cancer management.

This proposed project will focus on 3 specific aims:

1.Compare and complement 4FMFES-PET with FDG-PET and conventional imaging modalities, and evaluate how they improved prognosis and staging of ER+ advanced breast cancer patients;

2.Correlate 4FMFES/FDG uptake and staging with pathological data (histology, receptor status, grade), including distal biopsy metastases sampling;

3.Correlate 4FMFES/FDG uptake and staging with longitudinal outcomes (treatment response, progression-free survival, time-to-relapse) to determine which cohort of patient benefit most from 4FMFES.

This phase 2 trial will focus on stage III and IV breast cancer patients, which are the most likely to harbor tumor heterogeneity and thus to benefit from whole-body ER imaging with 4FMFES.

Expected outcomes of the study are, but are not limited to: 1) the correlation of 4FMFES-PET uptake with histopathological markers, notably ER immunohistochemistry (IHC) score; 2) the evaluation of progression-free survival and time-to recurrence versus 4FMFES-PET uptake threshold; 3) the correlation of treatment response (complete or partial response) with reduction of 4FMFES-PET uptake and number of assessable lesions at 6 and 18 months after the initial assessment; 4) a complementarity analysis of FDG-PET with 4FMFES-PET to allow for high sensitivity and high specificity characterization of breast cancer.

Upon completion, this project aims to demonstrate that 4FMFES-PET is an essential tool for breast cancer staging, prognosis, follow-up and therapy decisions. If our preliminary data forebode this project’s outcome, 4FMFES-PET will complement conventional imaging procedures to a point where breast cancer diagnosis will become unprecedently precise.

I3 Immunology, Signalling and Stem Cells

Alain, Tommy
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Bioengineering peak transgene production during viral oncolysis

Oncolytic viruses (OVs) target and kill tumour cells. They are promising immunotherapeutic agents for the treatment of cancer, with a global market value expected to reach (US) $6.4 billion within the next few years. The current cohort of OVs undergoing clinical trials (with the exception of reovirus) have been genetically engineered to encode transgenes (non-viral genes inserted into the viral genome) designed to enhance viral cytotoxic and immunostimulatory effects. These added payloads are essential to modulate the tumour microenvironment and to improve tumour detection and eradication by the immune system. However, these transgenes have not been rigourously optimized to maximize "payload expression per unit of virus", resulting ultimately in the delivery of "low efficiency" OVs to patients. We have identified three barriers to efficient transgene expression in bioengineered OVs: 1) Poor translation of the transgene mRNA; 2) decreased viral fitness due to expression cassettes that insert within essential viral genes or exceed viral genome size limits; or 3) sub-optimal promoter activity.Our **vision** in this proposal is to improve OV quality and thus efficacy of cancer virotherapy, by mitigating each of these three barriers. We propose that protein expression obtained by current transgene incorporation is sub-optimal because their mRNA transcripts lack critical viral translation control elements. **We hypothesize that we can optimize protein expression during OV infection by engineering transgenes with viral 5' untranslated regions (UTRs), codons adjusted for virus-specific translation, and viral promoter regions conferring high transcription**. In this proposal, we will identify, characterize and optimize viral cis-acting sequences and the genomic location of expression cassettes. We will improve transgene expression for two of the most promising and clinically tested OV platforms herpes simplex virus 1 (HSV1: Amgen, Sorrento Therapeutics, Virogin, Oncorus and others) and vaccinia virus (VV: Sillagen, Turnstone, Virocure and others). **AIM 1: Create high-translation transgene cassettes.** We will define the 5' UTRs of oncolytic HSV1 and VV from ribosome profiling data we developed as part of our CBCF/CCSRI Innovation Grant. From this existing data, we will identify and validate HSV1 and VV 5' UTRs associated with enhanced translation efficiency and optimize ORF codon usage**.** **AIM 2: Engineer transgene-expressing OVs with negligible effects on viral fitness and maximal transgene promoter activity.** We will explore the effect of genomic location on transgene expression by creating VV strains with transgenes placed in empirically defined genomic loci. We will identify genes that are highly transcribed at specific times during infection and map their promoter regions. We will then combine the codon optimization, top UTR enhancers and ideal location to create the optimized UTR-transgene-expressing viruses to test for therapeutic efficacy *in vivo* in Aim 3. **AIM 3: Demonstrate the superiority of our transgene-optimized OVs to current platforms.** We will use the hard to treat and clinically relevant 4T1 and EMT6 syngeneic models of breast cancer to quantify increased luciferase or transgene protein production within tumour tissues *in vivo* during infection with HSV1 and VV engineered to contain our optimal UTR-transgene cassette. **Impact:** By identifying viral regulatory elements and introducing these into the transgene expression cassette, we propose an innovative approach to overcome current barriers to therapeutic efficacy of OVs. To maximize benefits to cancer research and patients, we have already engaged end-users ready to adopt our enhancements into their OV platforms and move them to clinical trial.

I4 Novel Therapeutics

Ursini-Siegel, Josie
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Capitalizing on an oxidative stress response to sensitize poor outcome cancers to PARP inhibitors.

SIGNIFICANCE: Breast cancer is the second leading cause of cancer-related deaths in Canadian women. In addition to the physical and emotional impact on patients and their families, this disease also places a huge financial burden on the health care system. Breast cancers are stratified into distinct subtypes, which influence responsiveness to targeted therapies in addition to patient outcome. Unfortunately, triple negative breast cancer (TNBC) patients retain poor clinical outcome, which is attributed, in part, to the lack of targeted therapies. Representing 15-20% of all newly diagnosed cases, cytotoxic chemotherapies, such as doxorubicin, remain the standard of care to treat TNBC. These chemotherapeutics function, in part, by promoting the generation of reactive oxygen species (ROS). PARP inhibitors represent a promising class of compounds to treat women with TNBC. Many TNBCs are deficient in their ability to repair DNA damage, making them particularly susceptible to PARP inhibitors. Moreover, PARP inhibitors have been tested in clinical trials in combination with cytotoxic chemotherapies to treat TNBCs. Intriguingly, while response rates are low, small cohorts of patients do respond. Identification of the molecular mechanisms that contributes to responsiveness of TNBCs to PARP inhibitors is needed.

We may have uncovered a potential molecular basis to explain the poor efficacy of PARP inhibitors as monotherapies and identified strategies to increase their effectiveness in treating TNBCs when treated with particular cytotoxic chemotherapies. Of relevance to this proposal is the p66ShcA redox protein, which is variably expressed in breast cancer and stimulates ROS production in response to a variety of stress stimuli, including chemotherapy. We previously showed that p66ShcA is upregulated in many TNBCs. We now show that p66ShcA synergistically sensitizes TNBCs to a Doxorubicin/PARP inhibitor (Niraparib) combination, both in vitro and in vivo. This particular PARP1/2 inhibitor is currently being tested in breast cancer clinical trials. Mechanistically, we show that p66ShcA-induced sensitivity of TNBCs to this combination therapy is associated with an elevated oxidative stress response, leading to increased cell death.

HYPOTHESIS/OBJECTIVES: Given that p66ShcA promotes deleterious ROS when cells are exposed to therapy-induced stressors, **we hypothesize** that p66ShcA sensitizes TNBCs to Dox/PARPi therapy by potentiating mitochondrial dysfunction in cancer cells. **Our central objectives** are to examine the molecular mechanisms by which p66ShcA mediates this response and to identify therapeutic strategies that augment p66ShcA levels in TNBCs to maximize the number of patients that could benefit from a PARPi/Doxorubicin combination therapy. This will be achieved through the following aims:
Aim 1: To validate that p66ShcA is a predictive biomarker for responsiveness of TNBCs to Dox/PARPi therapy and to determine whether it also confers increased sensitivity of other cancer types (lung cancer, melanoma) to this drug combination.***Aim 2:*** To defined how p66ShcA perturbs mitochondrial dynamics and sensitizes TNBCs to Dox/PARPi therapy.***Aim 3:*** To improve therapeutic strategies to increase sensitivity of TNBCs to Dox/PARPi therapy.

EXPERTISE: Our team (Ursini-Siegel and Witcher) of researchers brings complementary expertise in p66ShcA signaling, breast cancer biology, mouse models, oxidative stress, PARP function, DNA repair and epigenetic control of gene expression. We secured collaborations with Dr. Julie St-Pierre who brings expertise in mitochondrial function and Drs. Morag Park and Peter Siegel, who established a TNBC PDX cohort, to translate our findings into clinical material. Finally, Dr. Claudia Klienman will bring valuable computational expertise to this study and Dr. Wilson Miller will be essential for us to translate these findings into the clinic. Our study may pave the way for studies that allow reactivation of oxidative stress pathways in response to chemotherapeutics and give new hope to the application of PARP inhibitors to treat TNBC patients, and potentially even other malignancies.