**Abstract Title:** Validation of an enzyme-linked immunosorbent assay for assessing serum B-cell maturation antigen levels in patients with multiple myeloma

**Introduction:** Multiple myeloma (MM) is a cancer that results from the abnormal proliferation of plasma cells which normally reside in the bone marrow and secrete antibodies. Patients generally present in a premalignant stage, such as monoclonal gammopathy of undetermined significance (MGUS) or smouldering MM (SMM). Currently, serum monoclonal antibody levels are used to monitor patients for progression from MGUS or SMM to full-blown MM as well as their response to treatment. Unfortunately, patients with non-secretory MM cannot be monitored using monoclonal antibody levels and require more invasive procedures to monitor their disease status. Serum B-cell maturation (sBCMA) is an emerging alternative biomarker with the potential to monitor disease status in secretory and non-secretory MM patients. We are looking to: (i) validate an sBCMA enzyme-linked immunosorbent assay (ELISA) for use with the automated EUROIMMUN Analyzer I instrument, and (ii) assess the stability of sBCMA in stored patient serum samples.

**Methods:** Validation of the EUROIMMUN instrument was done by generating three sets of pooled patient serum as quality control (QC) samples. The QC samples were run on the EUROIMMUN instrument as per the ELISA kit manufacturer’s instructions and a standard curve was generated using the BCMA standards provided with the kit. Coefficients of variation (CVs) of the QC samples and recoveries of the standards were calculated to assess precision and accuracy, respectively, of the instrument. BCMA stability was assessed by performing ELISAs on fresh and stored serum samples.

**Results:** The EUROIMMUN’s precision was acceptable, with both the within-run and between-run CVs meeting our target of 20% or less. In terms of accuracy, the instrument generally met our target recovery range of 100% ± 20%, although the recovery of the lower concentrations was outside of this range. Meanwhile, performing the ELISA manually resulted in improved recovery of the standards. Assessment of BCMA stability is still ongoing, as there were inconsistencies and issues with the quantification of the samples.

**Discussion:** The EUROIMMUN Analyzer I provides acceptable precision when performing a BCMA ELISA. However, issues with our BCMA stability experiment and the out-of-range recoveries indicate that more work needs to be done in order to improve the accuracy of the instrument.
**Background:** There is increasing support for distinguishing between the multiple dimensions of sex and gender which exist. However, most survey data continue to classify respondents according to a single, unclearly defined dimension of sex/gender, which can have the effect of masking critical health findings. Hysterectomy-corrected reproductive cancer research has demonstrated how deleterious this assumption can be, having revealed an underestimation of cervical cancer rates among Black and elderly women. Sex biases within animal models have also contributed validity issues in reproductive cancer research. Canines, in particular, are potentially at high risk of unacknowledged proxy measurement of sex and cancer risk due to the high prevalence of hysterectomy within this population. The purpose of this study is to examine the validity of administrative sex/gender as a proxy for uterine and ovarian status and assess whether and how this may vary across age and ethnoracial groups. Furthermore, we aim to identify and connect key stakeholders to advance sex/gender.

**Methods:** Sensitivity and specificity were calculated to estimate the validity of administrative sex/gender as a proxy for uterine status and ovarian status across age and ethnoracial groups using data from two large data sets from the U.S. – the National Health and Nutrition Examination Survey (NHANES) and the Behavioral Risk Factor Surveillance System (BRFSS) study. Positive and negative predictive values for the populations have also been calculated. A literature review was conducted to investigate canine hysterectomy research, with attention to the measurement of sex and reproductive cancer risk in canine species. A visual diagram of key stakeholders in reproductive cancer was constructed.

**Results:** Initial work has been conducted and analysis is currently in progress, but we are confident that results will be ready to be presented on April 7, 2021. We expect to generate base information that can be incorporated by reproductive cancer researchers, including the total proportion of the U.S. population with hysterectomy and oophorectomy among those classified female for survey sex/gender identity, as well as across intersections of race/ethnicity and age.

**Discussion:** Results from this study will contribute to promoting proxy evaluation to advance understanding of sex and gender multidimensionality.

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**Abstract Title:** Cell Cycle Control in Cancer Chemotherapy

**Introduction:** Cancer therapies that target specific molecules have shown positive benefits with limited side effects. One drug in this category is Palbociclib, which was developed as treatment for ER+/HER2- breast cancer. Palbociclib, a selective inhibitor of cyclin dependent kinases 4 and 6 (Cdk4/6), enables the activity of the tumor suppressor protein Rb. Inhibition of Cdk4/6 is a prominent area of interest as there is extensive research linking its overactivity to dysregulated breast cell proliferation. While addition of Cdk4/6 inhibitors to hormonal therapies has demonstrated positive results in clinical trials, the novelty of this drug raises many questions. One such question is how to manage the challenge of predicting efficacy in patients as well as the potential for resistance. Previous research in our lab has revealed Cdk inhibitor protein p27 shares redundant cell cycle arrest functions with Rb, with important interactions between the two. We hypothesize Rb-dependent activation of p27 is critical to Palbociclib as its efficacy depends on enabling Rb function.

**Aims:**
1. Identify genes essential to Palbociclib mediated arrest of ER+/HER2- breast cancer cells engineered to rely on p27 related cell cycle arrest
2. Define a signalling pathway utilizing Rb-related stabilization of p27. Develop biomarkers that can predict Palbociclib efficacy and resistance from these findings

**Methods:** To test this hypothesis we will identify essential genes by conducting a genome-wide CRISPR knockout screen on ER+/HER2- breast cancer cells modified to be reliant on p27 related cell cycle arrest. Then we can mechanistically investigate p27-Rb interactions that govern cell cycle arrest. With this, potential biomarkers for Palbociclib efficacy and resistance can be identified and cross referenced against stored tissue samples of patients treated in similar fashions.

**Significance:** With characterization of Rb-p27-Cdk activity in cells, we can identify critical biomarkers to accurately predict Cdk4/6 inhibitor efficacy and potential for developed resistance.
**Abstract Title:** Effects of mismatch repair deficiency on MHC class I expression and interferon signalling in neuroblastoma and melanoma cells

**Introduction:** Highly immunogenic tumors often have higher tumor mutational burden (TMB) and respond better to immune checkpoint blockade immunotherapy. DNA mismatch repair (MMR) deficiency is a common cause of increased TMB and can be induced in cancer cells by knocking out components of the DNA MMR pathway, such as MLH1. By inducing MMR deficiency in cells, it may be possible to increase the immunogenicity of tumors and sensitize them to immunotherapy. However, it is currently unknown how antigen presentation in tumor cells is affected by induced MMR deficiency. Recent work in our lab showed that the induction of MMR deficiency in Neuro-2a mouse neuroblastoma cells resulted in an increase in the surface expression of MHC class I. Here, we aim to further elucidate which components of the MHC-I and interferon signalling pathway are affected in tumors with induced MMR deficiency. We hypothesize that MMR-deficient cells express higher levels of MHC class I molecule components and have increased activation of the interferon response pathway compared to their MMR-proficient counterparts.

**Methods:** Neuro-2a neuroblastoma and B16-F10 melanoma cells, as well as their respective clones with MLH1 gene knockout to induce MMR deficiency, were used. MMR-deficient (dMMR) and MMR-proficient (pMMR) cells were cultured for two different time periods (8 weeks and 14 weeks for Neuro-2a cells, 8 weeks and 16 weeks for B16-F10 cells) to allow for the accumulation of mutations. Cells were lysed and intracellular levels of MHC class I, TAP1, JAK1, JAK2, phospho-JAK1, and phospho-JAK2 were determined by western blot analysis.

**Expected Results:** We expect to see higher levels of MHC class I, TAP1, JAK1, JAK2, phospho-JAK1, and phospho-JAK2 in dMMR cells from both cell lines compared to pMMR cells. In addition, we expect that cells cultured for longer periods of time (14 weeks for Neuro-2a, 16 weeks for B16-F10) will also have higher levels of MHC class I, TAP1, JAK1, JAK2, phospho-JAK1, and phospho-JAK2 compared to cells cultured for a shorter duration of time.

**Discussion:** Findings from this project will help characterize the response of cancer cells to induced MMR deficiency and may help further our understanding of potential mechanisms that contribute to higher immunogenicity in MMR-deficient tumors. This project contributes to the exploration of induced MMR deficiency in tumor cells as a strategy to improve immune checkpoint blockade.
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Abstract Title: Carboplatin induces Wnt Signalling in Angiogenesis-Mediated Ovarian Cancer Resistance

Introduction: Despite significant improvements in the prognosis of many solid cancer types, survival rates post-high grade serous ovarian cancer (HGSOC) diagnosis have remained low over the last 30 years. Chemotherapy resistance is prevalent and particularly common in recurrent disease. Both the Wnt/β-catenin developmental pathway and angiogenic signalling are known to be upregulated in chemoresistant HGSOC cells, contributing to resistance to its standard treatment: carboplatin. However, the connection between Wnt and angiogenic signalling in the development of resistance is yet to be elucidated. We thus hypothesize that carboplatin treatment induces the Wnt pathway, changing angiogenic factor expression in a mechanism that promotes the survival of epithelial ovarian cancer cells.

Methods: To investigate Wnt induction, we used immunofluorescence microscopy to identify nuclear β-catenin staining in HGSOC tissues before and after treatment with carboplatin. Additionally, carboplatin induction of the Wnt pathway in HGSOC was measured by culturing cells from the COV362 line with carboplatin treatments (0, 25, 50μg/mL). qPCR analysis of Wnt pathway proteins AXIN2, WISP1, and CCND1 was conducted. As well, pro-angiogenic factor expression in the cells will be quantified using qPCR analysis.

Results: We expect to see increased β-catenin nuclear reactivity in chemotherapy-treated tissues. It is also expected that the Wnt pathway will be upregulated after carboplatin treatment, as shown by an increase in key Wnt pathway gene mRNA levels. Furthermore, it is expected that mRNA levels will increase for pro-angiogenic genes in response to Wnt pathway activation.

Discussion: The results of this study can contribute towards identifying the mechanism of chemoresistance in HGSOC, integrating current knowledge about Wnt/angiogenesis induction. Results may indicate novel targets for tumour surveillance and therapy and suggest future avenues for research in other tumours treated with carboplatin.