Presenter's Name: Cheong, Ian

Additional Authors: Edmond P, Hildebrand D, Yang L, Rutledge A

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.

POSTER PRESENTATIONS 1
1B: CANCER BIOLOGY 1

Presenter's Name: Good, Hayley

Additional Authors: Shin AE, Zhang L, Meriwether D, Reddy ST, Wang TC, Asfaha S

Abstract Title: Aspirin Prevents Tuft Cell-Derived Colitis-Associated Cancer in a PGE2 and Phospho-Akt Dependent Manner

Introduction: Inflammatory bowel disease (IBD) is a major risk factor for colorectal cancer (CRC). Despite the link between inflammation and cancer, the mechanism by which colitis leads to cancer is unknown. Dclk1 is a marker of tuft cells. We previously showed that Dclk1+ cells are quiescent, long-lived, and remain resistant to proliferation even upon mutation of the tumor suppressor APC. Following induction of colitis, however, APC-mutated tuft cells transform into cancer-initiating cells, but the mechanism by which this occurs is unknown. Interestingly, Dclk1+ cells express high levels of cyclooxygenase (COX)-1 and -2, the direct enzyme targets of Aspirin. Aspirin is also a well-known chemopreventative drug in CRC. Therefore, we aimed to determine the effect of Aspirin on Dclk1+ cell-derived colitis-associated cancer (CAC).

Methods: Dclk1CreERT2;APCfl/fl;R26mTmG mice were treated with tamoxifen to induce APC-loss and GFP expression in Dclk1+ cells and their progeny. This was followed by administration of DSS to induce colitis and daily treatment with Aspirin or vehicle. Sixteen weeks post-tamoxifen, colonic tumor number and size were examined. Acute colonic tissue was collected for analysis of COX-derived prostaglandins by liquid chromatography-mass spectrometry (LC-MS). To investigate how Aspirin may be influencing tumorigenesis, we focused on PGE2, a key prostaglandin in CRC, and phospho-Akt, a downstream mediator of PGE2. The effects of Misoprostol (PGE analogue) and SC79 (Akt activator) on Dclk1+ cell-derived tumorigenesis were examined. Colonic tissue was collected 3 weeks post-DSS and analyzed for GFP+ cells by fluorescence microscopy.

Results: Aspirin significantly reduced the number of Dclk1+ cell-derived colonic tumors. LC-MS analysis revealed that prostaglandins, including PGE2, were significantly elevated in DSS-colitis and reduced upon Aspirin treatment. Treatment with vehicle, Misoprostol, or SC79 during DSS showed GFP-traced crypts with normal morphology, however, treatment with both Misoprostol and SC79 resulted in GFP-traced dysplastic lesions.

Conclusion: Our data suggests that Aspirin inhibits the initiation of CAC through downregulation of PGE2, which synergizes with Akt signaling to promote tumorigenesis during colitis.

Presenter's Name: Khando, Pema

Additional Authors: Bauer GB

Abstract Title: Measurement Bias in Sexual and Reproductive Cancer in Humans and Canines: A Comparative Assessment

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.

POSTER PRESENTATIONS 1
1B: CANCER BIOLOGY 1

Presenter's Name: Morin, Monique

Additional Authors: Brackstone M, Burton J, Lynn K

Abstract Title: Re-setting the breast microbiome to lower inflammation and risk of cancer

Introduction: As of 2021, breast cancer is the most commonly diagnosed cancer and the leading cause of cancer death worldwide. Despite major advances in understand and treating breast cancer, there are still a multitude of "environmental factors" that remain to be understood. One factor of interest is the microbiome of breast tissue. The population of bacteria found in breast cancer tissue has been shown to be more pathogenic than bacteria found in healthy control tissue. The pathogenic bacteria species were known to induce DNA damage and promote inflammation, two hallmarks of cancer. Assuming that there is a link between the pathogenic bacteria and cancer progression and/or induction, we are interested in exploring the possibility of reducing the inflammatory microbiota and consequently reducing the risk of breast cancer. Probiotics have recently emerged as a promising treatment for inflammation of the breast. A group of Spanish researchers found that ingestion of probiotic lactobacilli was able to treat inflammatory breast disease in women. We will investigate whether women at high-risk of breast cancer also have inflammatory microbiomes and if we are able to re-set their microbiomes using probiotic lactobacilli. Probiotics have also proven to be promising in sequestering and eliminating environmental toxins, such as heavy metals and pesticides, in pregnant women. Environmental toxins are known to stimulate breast cancer induction and production, so we will also investigate the ability of probiotics to reduce the levels of toxins in our study participants.

Hypothesis: We hypothesize that the breast microbiome of women at risk of cancer has the same profile as women with cancer, and that oral administration of probiotic lactobacilli can re-set this to one found in healthy women. We also hypothesize that the probiotic administration will be able to lower inflammatory cytokine and environmental toxin levels.

Methods: To test this hypothesis, 20 women at high risk of developing breast cancer and 20 healthy control women will be randomized to taking the oral probiotic lactobacilli or placebo for 90 days. Participants will have urine, blood, and breast samples taken at day 0, day 90, and day 120. 16sRNA Illumina Miseq, Luminex assay of inflammatory markers, liquid chromatography-mass spectrometry (LC-MS) will be used to identify bacteria populations, cytokine levels, and pesticide levels respectively. The plasma from the blood samples will be used to measure the levels of cytokines using Luminex immunoassay kits. The urine samples will also be tested for heavy metals using an Element 2 high resolution inductively coupled plasma mass spectrometry (ICP-MS) and for pesticides using LC-MS.

Significance: This study could identify if an aberrant microbiota is linked to women at high-risk of breast cancer and can be reverted back to a healthy microbiome. If such a link exists, we can further follow women at high-risk of breast cancer and see if probiotics can delay the onset of breast cancer. This study will also help us identify metabolites and inflammatory markers associated with an aberrant microbiota and if probiotics are able to reduce these levels.

Presenter's Name: Ratushny, Liam

Additional Authors: Howlett C, Dick F

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.

POSTER PRESENTATIONS 1
1B: CANCER BIOLOGY 1

Presenter's Name: Rosic, Damir

Additional Authors: McCord, C

Abstract Title: Characterizing Human Papillomavirus Associated Oral Epithelial Dysplasia

Background: The role of high risk (HR) Human papillomavirus (HPV) has been well documented in the development and progression of oropharyngeal squamous cell carcinoma and cervical cancer. In fact, greater than 70% of oroparyngeal cancer cases are believed to be associated with HR HPV. Currently, literature projects the association of HPV with oral cancers as being approximately 6%, HR HPV infection has been identified in a subset of oral dysplastic lesions. Due to the rarity of this lesion, literature describing HPV associated OED is limited.

Objectives: To identify and characterize cases of HPV associated oral epithelial dysplasia that are p16 positive and show E6/E7 expression by RT-PCR. To determine the utility and accuracy of tissue-based HPV 16 E6 antibody for HR HPV in the diagnosis of HPV associated oral epithelial dysplasia.

Methods: This is a retrospective study of archived tissues submitted to the Oral Pathology Diagnostic Service in the Division of Oral Pathology, at Western University. Consecutive cases of carcinoma-in situ (CIS) and/or Bowenoid dysplasia have been identified from the Oral Pathology database for the years 2002-2019. Approximately 115 cases have been identified which show histopathologic features of HPV infection. Cases that met the acceptability criteria were stained for p16 (Roche) using standard immunohistochemical techniques. Cases that are deemed diffusely positive for p16 and/or HPV E6 will then be subjected to RT-PCR to detect and quantify HPV 16 E6 mRNA. We have stained our p16 positive cases with E6 antibody (Abcam) in order to evaluate its possible utility as a diagnostic marker for HPV.

Results: Preliminary results show approximately 70 cases that have been deemed p16 positive and these will be subjected to RT-PCR to detect and quantify HPV 16 E6 mRNA. These cases show a strong predilection for men and seem to affect sites such as the tongue and the floor of the mouth more often. Our results regarding E6 antibody staining were not helpful as the staining turned out to be diffuse and non-specific. Further results are pending.

Presenter's Name: Xu, Yili

Additional Authors: Xu Y, Figueredo R, Krishnamoorthy M, El-Hajjar M, Gerhardt L, Maleki S

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.

POSTER PRESENTATIONS 1
1B: CANCER BIOLOGY 1

Presenter's Name: Zakirova, Komila

Additional Authors: Zakirova K, Passos D, Dick F

Abstract Title: Identification of molecular mechanisms of spheroid dormancy in epithelial ovarian cancer

Epithelial ovarian cancer (EOC) generally presents at a late stage and is the most lethal of the gynecologic malignancies. A common symptom of advanced EOC is the peritoneal localization of metastasis. Primary tumors exfoliate cells into the ascites where they form multicellular aggregates known as spheroids. These spheroids carried by the ascites fluid further attach and invade secondary sites within the abdomen. Spheroids evade numerous antitumor therapeutics, which target metabolically active cells, by withdrawing from the cell cycle into a dormant state and are the principal source of residual disease relapse in EOC patients. The persistence of drug-resistant cancer cells remains the major challenge in successful treatment of EOC and highlights the importance of elucidating the molecular mechanisms of dormancy.

Previous findings from our laboratory show that the DYRK1A kinase plays a crucial role in the growth arrest of ovarian cancer cells. Loss of DYRK1A activity, via genetic deletion or chemical inhibition, hindered spheroid formation in a panel of EOC cell lines that were cultured in the model system of cellular dormancy. Chemical inhibition of DYRK1A also resulted in improved sensitivity to carboplatin, indicating it may have therapeutic potential in the disease treatment. These in vitro findings suggest that EOC cells deficient for DYRK1A kinase are unable to exit the cell cycle and form dormant spheroids, and consequently are susceptible to chemotherapy. I will investigate whether this phenotype of DYRK1A loss can be recapitulated in vivo in mouse models of metastasis.

Dyrk1A is a dose dependent gene, and either an excess or deficit in expression is detrimental for the normal functioning of the brain and body. Altered expression of DYRK1A has been shown to be lethal during early development. The effects of DYRK1A deficiency and its relevance in a fully developed adult are yet to be established but are highly important to understand the side effects of DYRK1A inhibition.

POSTER PRESENTATIONS 1
1B: CANCER BIOLOGY 1

Presenter's Name: Zhang, Ruo Hao

Additional Authors: Khan, ZA

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.