UNIVERSITY of GUELPH

INSTITUTE FOR COMPARATIVE CANCER INVESTIGATION

15th Annual ICCI

Cancer Research Symposium

Thursday May 25, 2023

Zoom Web Platform 9:00-4:00

Introductory Remarks

Welcome to the 15th annual Guelph ICCI Cancer Symposium! This meeting is an opportunity to bring together cancer researchers from across campus and regional collaborators. Topics range from basic science through to clinical application. We are very grateful to the amazing group of speakers and poster presenters who will be sharing their findings with us today. Dr. Amy LeBlanc is the 2023 Arthur Willis Distinguished speaker and will be giving the keynote address at 12:50 p.m.

In the past 15 years we have seen relationships and collaborations develop that were made possible by these interactions and we hope that this year's meeting will spark new collaborations and ideas.

This symposium is made possible by funding from the Arthur Willis Visiting Professorship in Canine Oncology and support from the OVC Dean's office.

Drs Geoff Wood and Michelle Oblak
Pathobiology and Clinical Studies, University of Guelph
ICCI Co-Directors

Administrative Support and Research Funding:

Many thanks to Deirdre Stuart for organizing this event and setting up the virtual site, the OVC administrative assistants and communications team for their extensive help with information dissemination, and Zoom Web Services and our University of Guelph Information Technology team for help with the virtual meeting platform.

The research projects presented here and the trainees performing these studies were collectively supported by grants, scholarships and contracts from: Allard Research Chair Start-up Fund, Art Rouse Memorial Scholarship, Brock Doctoral Scholarship, Canadian Institutes of Health Research, Cancer Research Society, Companion Animal Health Fund, Emerging Leaders of the Americas Scholarship, Ethel Rose Charney Scholarship, Federal Rural University of Amazonia, Memorial University of Newfoundland, Mitacs Globalink, National Science and Technology Council (CONACYT) of Mexico, Natural Sciences and Engineering Research Council of Canada, Ontario Institute of Cancer Research, Ophir Loyola Hospital, OVC Pet Trust, Saskatchewan Health Research Foundation, Terry Fox Research Institute,The Smiling Blue Skies Cancer Fund and Vanier Canada Graduate Scholarship.

ICCI 15th Annual Cancer Research Symposium, Thursday May 25th, 2023

Morning Session

9:00-9:05 Welcome and Introductory Remarks (Michelle Oblak and Geoff Wood)

9:05-9:45 Guest Speaker. Moderator: Michelle Oblak

Metabolic modulation by avocado-derived bioactives improves cancer outcome
Dr. Paul Spagnuolo; Department of Food Science, University of Guelph

9:45-10:25 Short talks from abstracts. Moderator: Michelle Oblak

1. *MicroRNA profiles determined from lymph node cytology slides to immunophenotype and prognosticate canine multicentric lymphoma*
   Dante Meza; Department of Pathobiology, University of Guelph

2. *Fc3TSR remodels the tumor microenvironment to enhance the uptake and efficacy of chemotherapy and immunotherapy in a murine model of pancreatic ductal adenocarcinoma*
   Bianca Garlisi; Department of Biomedical Sciences, University of Guelph

3. *Investigating alterations in canine melanoma cell glucose metabolism under hypoxic conditions (Poster)*
   Keren Chernov; Department of Biomedical Sciences, University of Guelph

4. *Development of canine appendicular osteosarcoma in vitro metastasis assay pipeline (Poster)*
   Emma Vanderboon; Department of Pathobiology, University of Guelph

10:25-10:40 Biobreak – Please take this opportunity to visit our posters at https://icci.uoguelph.ca/icci-annual-symposia /2023-icci-symposium-posters/

10:40-11:20 Guest Speaker. Moderator: Geoff Wood

Round cells and radiation
Dr. Valerie Poirier; Radiation Oncologist, Animal Cancer Centre, University of Guelph

11:20-12:00 Short talks and poster presentations from abstracts. Moderator: Michelle Oblak

5. *Expression and function of the EphA2 receptor in human and canine melanoma*
   Shabnam Abdi; Department of Small Animal Clinical Sciences, Western College of Veterinary Medicine, University of Saskatchewan

6. *Expression of receptor tyrosine kinases in canine appendicular osteosarcoma: prognostic significance, and potential as therapeutic targets*
   Rachael Speare; Department of Pathobiology, University of Guelph

7. *Clinical outcome of 27 dogs with infiltrative lipoma treated with radiation therapy: A multi-institutional retrospective study*
   Dr. Arata Matsuyama; Medical Oncologist, Department of Small Animal Clinical Sciences, Western College of Veterinary Medicine, University of Saskatchewan

12:00-12:50 ***Lunch Break and Poster Viewing***

Afternoon Session

12:50-2:00 **Keynote Speaker.** Moderator: Geoff Wood

Comparative oncology efforts in canine osteosarcoma: A translational patient model for humans

Dr. Amy LeBlanc; Senior Scientist and Director of the CCR Comparative Oncology Program, National Cancer Institute, National Institutes of Health

2:00-2:50 Short talks from abstracts. Moderator: Alicia Viloria-Petit

8. *Re-expression of miR-200c/141 in triple-negative breast cancer cells suppresses Notch signalling and impairs tumour growth and metastasis*
   Megan Vaz; Department of Biomedical Sciences, University of Guelph

9. *Investigating ABC proteins in triple-negative canine mammary cancer*
   Raissa Melo de Sousa; Federal Molecular Biology Laboratory, Institute of Biological Sciences, Federal University of Pará

10. *Nck adapter proteins regulate breast cancer cell metastasis and invasion*
    Erka Shata; Department of Molecular and Cellular Biology, University of Guelph

11. *Identification of lysyl oxidase as an adipocyte-derived inducer of morphological changes in MDA-MB-231 cells*
    Cassidy Van Stiphout; Department of Biomedical Sciences, University of Guelph

2:50-3:10 Biobreak – Please take this final opportunity to visit our posters at https://icci.uoguelph.ca/icci-annual-symposia /2023-icci-symposium-posters/

3:10-4:00 Guest Speaker. Moderator: Alicia Viloria-Petit

The ups and downs and ups of drugs developed as angiogenesis inhibitors for cancer treatment

Dr. Robert Kerbel; Senior Scientist, Sunnybrook Research Institute

4:00-4:05 Closing Remarks

KEYNOTE PRESENTATION

12:50 – 2:00 p.m.

Dr. Amy LeBlanc, DVM, DACVIM

Senior Scientist and Director of the CCR Comparative Oncology Program, National Cancer Institute, National Institutes of Health

Comparative oncology efforts in canine osteosarcoma: A translational patient model for humans

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Dr. Amy LeBlanc is a board-certified veterinary oncologist, Senior Scientist and Director of the CCR Comparative Oncology Program at the National Cancer Institute, NIH. In this position, she directly oversees and manages the operations of the Comparative Oncology Trials Consortium (COTC), which designs and executes clinical trials of new cancer therapies in tumor-bearing pet dogs.

Dr. LeBlanc is a graduate of Michigan State University, holding both B.S. and D.V.M. degrees. She completed a rotating internship in small animal medicine and surgery at Texas A&M University and a residency in companion animal oncology at Louisiana State University. She is board-certified by the American College of Veterinary Internal Medicine.

Prior to her appointment at NIH, Dr. LeBlanc was an Associate Professor with tenure and Director of Translational Research at the University of Tennessee College of Veterinary Medicine (CVM) and UT Graduate School of Medicine (GSM). Dr. LeBlanc's group at the University of Tennessee published the first comprehensive studies describing molecular imaging of dogs and cats using PET/CT, focusing on the forward and back-translation of ^{18}F-labelled radiopharmaceuticals.

Dr. LeBlanc has given numerous invited lectures on the inclusion of companion animals in imaging-based translational research and the value of comparative oncology in drug and imaging agent development.Her program also provides support to several extramural NCI-funded initiatives including the Integrated Canine Data Commons and Cancer Moonshot-funded canine immunotherapeutic clinical trials conducted under the PRECINCT network.

Past ICCI Symposium Arthur Willis Distinguished Speakers

2022	Elizabeth Murchison	2017	William Eward	2013	David Argyle
2021	Lisa Forrest	2016	Jaime Modiano	2012	Timothy Fan
2019	David M. Vail	2015	Nicola Mason	2011	Cheryl London
2018	Daniel Gustafson	2014	Deborah Knapp	2010	Matthew Breen

GUEST SPEAKER:

9:05-9:45

Metabolic modulation by avocado-derived bioactives improves cancer outcome

Dr. Paul Spagnuolo, BSc, MSc, PhD; Department of Food Science, University of Guelph

Dr. Spagnuolo joined the Department of Food Science as an Associate Professor in 2016 after holding an Assistant Professor position at the School of Pharmacy, University of Waterloo. His research is focused towards investigating the anti-cancer effects of food-derived bioactive compounds, specifically nutraceuticals that target and destroy leukemia and leukemia stem cells. These compounds are then utilized as molecular probes to further investigate the pathways that control cancer cell survival and death. Overall research objectives are to identify new anti-cancer neutraceutical compounds and determine their regulation in apoptosis and other forms of cell death.

GUEST SPEAKER:

10:40-11:20

Round cells and radiation

Dr. Valerie Poirier, PhD**;** Radiation Oncologist, Mona Campbell Animal Cancer Centre, University of Guelph

Dr. Poirier was born in Quebec and graduated from University of Montreal in 1998. She started working at the University of Wisconsin in 1999 where she acquired her Diplomate of the American College of Veterinary Internal Medicine (Oncology) in 2003. From there, she moved to Switzerland and began work at the University of Zurich in 2003 working as a medical and radiation oncologist and acquired Diplomate status of the American College of Veterinary Radiology (Radiation Oncology) – Medical and Radiation Oncologist in 2006. In 2007 she moved to Australia and joined the team at Brisbane Veterinary Specialist Centre and helped built the first radiation facility for pets in the southern hemisphere. She moved to Guelph in August 2012 and is part of the Animal Cancer Centre as a Radiation Oncologist.

GUEST SPEAKER:

3:10-4:00

The ups and downs and ups of drugs developed as angiogenesis inhibitors for cancer treatment

Dr. Robert Kerbel, PhD; Senior Scientist, Sunnybrook Research Institute

Dr. Kerbel is Senior Scientist at the Sunnybrook Research Institute, and a Professor in the Dept. of Medical Biophysics, University of Toronto; he is formerly a Tier 1 Canada Research Chair recipient (2001-2015). His overall main research interest is devising new cancer treatment strategies having improved efficacy and reduced toxicity for the treatment of metastatic disease based mainly on exploiting aspects of the tumor microenvironment (TME). This culminated in his translational studies of low dose 'metronomic' chemotherapy including in combination with targeted antiangiogenic drugs. He has been studying tumor angiogenesis and antiangiogenic therapy since 1990 and is a leader in the field.

Other major contributions include development of improved preclinical investigational therapeutic models in mice involving early stage or advanced metastatic disease, uncovering mechanisms by which antiangiogenic drugs increase chemotherapy efficacy, and elucidating mechanisms of intrinsic or acquired resistance to antiangiogenic drugs, and more recently, immune checkpoint antibodies. One of his recent research interests is assessment of the significance of 'vessel co-option' in tumors on response to antiangiogenic drugs; another is assessing combination therapy utilizing immune checkpoint inhibitors with VEGF targeting antiangiogenic drugs, or metronomic chemotherapy, using new mouse tumor models for immune therapy his lab is developing. His studies involve extensive collaborations with both academia and industry. He has authored or co-authored 452 papers, has an H factor of 125, and given 900 invited lectures around the world.

His research programs over the years have been supported by long term grants from several funding agencies including the Canadian Institutes for Health Research (CIHR), the Canadian Cancer Society Research Institute (CCSRI), Worldwide Cancer Research (WCR), and the National Institutes of Health (NIH), USA, among others, as well as a number of sponsored research agreements with industry. Among the awards he has received include the 2004 Canadian Cancer Society Robert Noble Award for Excellence in Cancer Research, the Breast Cancer Research Award from the European Institute of Oncology in 2008, a Man of Distinction Honor by the Israel Cancer Research Fund in 2011, and the Colin Thomson Memorial Medal for achievements in cancer research from Worldwide Cancer Research in 2013.

SHORT TALKS FROM SUBMITTED ABSTRACTS

9:45-10:25 Session One

MicroRNA profiles determined from lymph node cytology slides to immunophenotype and prognosticate canine multicentric lymphoma

Dante Meza[1], Geoff Wood[1], Dorothee Bienzle[1], Anthony Mutsaers[2], Paul Woods[3], Darren Wood[1]*. 1. Department of Pathobiology, University of Guelph; 2. Department of Biomedical Sciences, University of Guelph; 3. Department of Clinical Studies, University of Guelph

Lymphoma is a heterogeneous disease that represents one of the most diagnosed neoplasms in dogs. It has a highly variable prognosis and multicentric lymphoma is the most frequent clinical presentation. The current standard-of-care treatment consists of a multi-agent chemotherapy protocol. Remission is obtained in most cases, although the disease-free interval is variable, and relapse inevitably occurs. Several classification schemes have been used to differentiate subtypes of lymphoma; however, it is difficult to predict which patients will respond well to treatment. MiRNAs are short molecules of non-coding RNA with an important role in multiple biological functions. They are implicated in the development of lymphoma and expression is often correlated with tumor immunophenotype, response to treatment, risk of metastasis, and overall survival. The goal of this study is to characterize the miRNA profile from lymph node cytology samples collected during the diagnostic workup, and to use this profile to classify the subtype of lymphoma and predict treatment response and prognosis. Our lab has identified a group of miRNAs that are differently expressed depending on the lymphoma immunophenotype; miR-130b-3p, miR-181a, b, c and miR-182-5p in association with T-cell lymphoma, and miR-18a, miR-19a, b, miR25-5p, miR-29b-3p, miR-34a-5p and miR-99a-5p with B-cell lymphoma. Our future goals include identification of miRNAs that have prognostic value and determination of a miRNA profile for use as a diagnostic test. If this information can be obtained from a single, stable, minimally invasive, routinely collected cytology sample, it would be a great advance in the management of canine lymphoma.

Fc3TSR Remodels the Tumor Microenvironment to Enhance the Uptake and Efficacy of Chemotherapy and Immunotherapy in a Murine Model of Pancreatic Ductal Adenocarcinoma

Garlisi, B[1], Aitken, C[1], Lauks, S[1], Stewart, S[1], Lawler, J[2], Petrik, J[1] 1. Department of Biomedical Sciences, University of Guelph; 2. Beth Israel Deaconess Medical Center and Harvard Medical School

Introduction*:* Pancreatic Ductal Adenocarcinoma (PDAC) has a poor survival rate due in part to late diagnosis when metastasis has occurred. Angiogenesis is the formation of new vessels from pre-existing vasculature, and is critical for tumour growth and metastasis. Tumours upregulate

expression of pro-angiogenic factors, stimulating rapid vessel formation, creating leaky vessels and high interstitial fluid pressures. This dysfunctional microenvironment greatly inhibits perfusion. Fc3TSR, derived from the 3TSR region of angiogenic inhibitor thrombospondin 1, has been seen to normalize vasculature in ovarian cancer in our lab, improving therapy uptake. In this study, we evaluated the ability of Fc3TSR to enhance delivery and efficiency of therapies into PDAC tumours.

Methods*:* We developed an orthotopic syngeneic murine model of PDAC whereby we injected 2.5x104 murine PDAC cells (KPC) into the pancreas. Tumours progressed for 14 days before administering Fc3TSR (0.158mg/kg) or PBS on day 14 and 21. For gemcitabine (GEM) chemotherapy, mice received daily low dose GEM, weekly high doge GEM, or PBS starting day 23. Another cohort of mice received checkpoint inhibitor PD-L1 (25ug), CTLA4 (25ug) or PBS on day 23 and 26. Mice were euthanized on day 30 and tumours and draining lymph nodes were collected and weighed.

Results*:* Fc3TSR reduced tumour size in mice compared to PBS but gemcitabine did not enhance this effect at either dosage. Checkpoint inhibitors after Fc3TSR reduce tumour size.

Conclusion*:* This data could provide evidence of the importance of normalizing vasculature before therapies to ensure effective delivery and improvement of immune responses in PDAC patients.

Funding: CIHR, OVC PhD Entrance Scholarship, Indigenous Graduate Scholarship

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Investigating alterations in canine melanoma cancer cell glucose metabolism under hypoxic conditions

Keren Chernov, Anthony Mutsaers*, Andrew Poon, Sarah Bernard, Peyton Tam. Biomedical Sciences, Ontario Veterinary College, University of Guelph

Canine melanoma is an aggressive cancer with high metastatic rates, resulting in poor outcomes. Treatment methods such as radiation and chemotherapy, such as carboplatin, show a limited response rate and most patients have a median survival of less than a year. Hypoxia in solid tumors induces pro-survival changes in metabolic pathways through effects such as increasing HIF-1 (hypoxia inducible factor 1). HIF-1 alpha promotes the Warburg effect, where cancer cells preferentially perform glycolysis to generate lactate for energy production regardless of oxygen availability. The PI3K/AKT/mTOR pathway, crucial for cell survival, growth, and metabolism, also enhances HIF-1 expression and contributes to metabolic adaptations that encourage treatment resistance. We examined whether hypoxia would increase anaerobic glycolysis in CML-1 canine melanoma cells and compared the effects of conventional platinum therapy using carboplatin alone and in combination with an mTOR inhibitor, rapamycin, on melanoma cell viability. CML-1 melanoma cells were cultured under either normoxic (room air, 21% O2) or hypoxic conditions (1% O2), and treated with either rapamycin or carboplatin, followed by metabolic analysis using the Agilent Seahorse XF Bioanalyzer to assess glycolytic activity. Preliminary results show increased glycolysis and decreased mitochondrial respiration in CML-1 cells under hypoxia compared to room air culture conditions. Inhibiting mTOR in CLM-1 cells at room air impacted

metabolism, and future experiments will further characterize will further characterize the effects of mTOR inhibition and carboplatin on cell viability during hypoxia.

Development of canine appendicular osteosarcoma in vitro metastasis assay pipeline

E. Vanderboon[1], A. Glogov[1], and C.R. Schott*[1]. 1. Department of Pathobiology, Ontario Veterinary College, University of Guelph

Despite aggressive treatment, 90% of dogs diagnosed with appendicular osteosarcoma are euthanized within two years of diagnosis due to metastasis. Metastasis is a multi-step process; functional assays mimicking these steps in vitro can help enhance our ability to interfere with this process. I am developing an in vitro assay pipeline to investigate mechanisms of metastasis and will characterize the performance of a panel of canine osteosarcoma cell lines. The panel consists of one commercial and nine primary cells lines, derived from both primary and metastatic tumors. I hypothesize that certain cell lines will perform steps of the metastatic cascade more efficiently than others in vitro. Metastasis is initiated when cells migrate and invade the adjacent tissue and enter the vasculature. To evaluate migration, scratch and transwell assays are performed. Next, invasive capacity will be evaluated using a transendothelial assay. Following vascular invasion, metastatic cells must survive transport within the blood, arrest within a vessel, and extravasate to additional sites where new colonies are formed. Capacity for anchorage independent survival will be measured using an anoikis assay, and a clonogenic assay will be used to evaluate colony formation. Thus far, the cell lines demonstrate variable capacity to migrate in the transwell assay, and plating efficiencies range from 8-68% after 7-14 days in the clonogenic assay. Assay optimization is ongoing. Performance within these assays will elucidate which cell lines are best to investigate specific steps of the metastatic cascade and the pipeline will be invaluable for future drug studies and mechanistic investigations.

Funding: OVC Pet Trust, Zoetis

11:20-12:00 Session Two

Expression and function of the EphA2 receptor in human and canine melanoma

Shabnam Abdi, Behzad M. Toosi. Department of Small Animal Clinical Sciences, Western College of Veterinary Medicine, University of Saskatchewan

Rationale: Melanoma is an aggressive and metastatic tumor in both humans and dogs. Despite advances in the treatment of melanoma, the prognosis of the more advanced stages of the disease is poor, therefore highlighting the need for more effective treatments. Erythropoietin-producing hepatocellular receptors (Eph receptors) are the largest family of receptor tyrosine kinases and are divided into two subfamilies (EphA and EphB). Accumulating evidence suggests that the EphA2 receptor is overexpressed in various human cancers and is involved in the regulation of cancer

invasiveness. Therefore, inhibition of this receptor alone or in combination with other therapies could conceptually improve cancer therapy. However, the role this receptor performs in promoting the fitness of human and canine melanoma is poorly studied. As a result, we hypothesized that EphA2 is expressed in canine and human melanoma, and that it regulates the proliferation, migration and invasion of melanoma cells.

Methods: EphA2 expression was assessed by Western blotting. To assess the functional relevance of EphA2 in melanoma cells, the expression of EphA2 was silenced in both canine and human melanoma cells using a specific shRNA. Control cells were transduced with a non-silencing shRNA and the EphA2 silencing was confirmed by Western blotting. The effects of EphA2 silencing on melanoma cell proliferation, invasion and migration were analyzed by Resazurin, Matrigel invasion and wound healing assays, respectively.

Results: EphA2 was strongly expressed in tested canine and human melanoma cells. Moreover, silencing of EphA2 resulted in decreased proliferation, colony formation, invasion and migration of both canine and human melanoma cells when compared with matching non-silenced controls.

Conclusion: Our data suggest that EphA2 is an important driver of the malignant behavior of both canine and human melanoma and provide the first functional evidence of a tumor-promoting role of EphA2 in canine melanoma.

Funding: Allard Research Chair in Oncology Start-up Fund, Saskatchewan Health Research Foundation (SHRF)

Expression of receptor tyrosine kinases in canine appendicular OSA: prognostic significance, and potential as therapeutic targets

Rachael Speare[1], Courtney R. Schott*[1], Robert A. Foster[1], Anthony J. Mutsaers[2], Geoffrey A. Wood*[1]. 1. Department of Pathobiology, University of Guelph; 2. Department of Biomedical Sciences, University of Guelph

Osteosarcoma is the most common bone tumour in dogs. Most patients die of metastatic disease within one year of diagnosis despite receiving standard of care (SOC) treatment. There is a lack of predictive biomarkers as well as novel candidates for targeted therapies. Receptor tyrosine kinases (RTKs) regulate cellular processes relevant to cancer progression and are targetable with tyrosine kinase inhibitors (TKIs). Few studies on RTK expression in canine osteosarcoma evaluate correlations with outcome. Clinical trials in dogs given TKI toceranib phosphate (TOC) have had limited success; these trials did not confirm expression of the molecular targets in the tumours before treatment. Immunohistochemistry for c-KIT, EGFR, IGFR, PDGFR-B, VEGFR-2, and p-AKT1 was performed for 126 cases from a canine appendicular osteosarcoma tissue microarray. Kaplan-Meier survival analyses for disease-free interval (DFI) and survival time (ST) were performed for each RTK in patients receiving SOC. PDGFRB, IGF1R, and p-AKT1 had immunolabelling in at least 20% of neoplastic cells in all cases. Over 70% of cases lacked c-KIT, EGFR, and VEGFR-2 immunolabelling. Nuclear p-AKT1 immunolabelling correlated with decreased ST ($p<0.05$). Low membranous PDGFR-B immunolabelling correlated with decreased

DFI ($p<0.05$) and ST ($p<0.05$). p-AKT1 immunolabelling correlated with a more aggressive disease course, which may represent enhanced p-AKT1 pathway activation secondary to enhanced RTK signalling. The primary target of TOC, c-KIT, is minimally expressed. Poor clinical efficacy of past TOC trials in canine osteosarcoma may be attributable to a lack of target protein expression. Pre-treatment quantification of target RTK expression is warranted for future TKI trials.

Clinical outcome of 27 dogs with infiltrative lipoma treated with radiation therapy: A multi-institutional retrospective study

A. Matsuyama[1], M.M. Turek[2], V.S. Meier[3], J. Lawrence[4], V.J. Poirier[5] 1. Department of Small Animal Clinical Sciences, Western College of Veterinary Medicine, University of Saskatchewan; 2. Department of Surgical Sciences, School of Veterinary Medicine, University of Wisconsin-Madison; 3. Division of Radiation Oncology, Small Animal Department, Vetsuisse Faculty, University of Zurich; 4. Department of Veterinary Clinical Sciences, College of Veterinary Medicine, University of Minnesota; 5. Department of Clinical Studies, Ontario Veterinary College, University of Guelph

Background: Radiotherapy (RT) has been utilized in the treatment of canine infiltrative lipoma; however, the existing evidence on its efficacy is limited to a single study published in 2000, comprising only 13 dogs.

Objective: To report the outcome of dogs with infiltrative lipoma treated with RT and identify prognostic factors.

Methods: This retrospective study included 27 dogs diagnosed with infiltrative lipoma. Dogs that underwent intensity-modulated radiation therapy (IMRT) or three-dimensional conformal radiation therapy (3D-CRT) and were followed for ≥2 years were included. Progression-free survival (PFS) was measured from RT initiation to progression/death. Statistical analyses included Kaplan-Meier, log-rank, Mann-Whitney U, and Cox proportional-hazards regression.

Results: Among 27 dogs, 21 received RT for gross disease, including 15 with recurrent infiltrative lipoma. Median gross tumor volume (GTV) was 154.2 cm^3 (range: 1.8-2534 cm^3), and median total RT dose was 51 Gy (range: 20-57 Gy). Five dogs (18.5%) experienced grade 3 acute skin toxicity (all received >51 Gy). Tumor shrinkage occurred in 9 dogs (42.9%) with gross disease, including 6 complete remissions. Overall median PFS was 1483 days (95% CI: 799-2167 days). Lower total RT dose and larger GTV were associated with shorter PFS (p=0.002, HR 0.894 [95% CI 0.831-0.961]; p=0.021, HR 1.002 [95% CI 1.000-1.003], respectively). Dogs with disease progression had significantly larger GTV (median 769.6 cm^3 vs. 108.9 cm^3, p=0.005).

Conclusion: RT provides long-term control for canine infiltrative lipoma. Higher total radiation doses may improve treatment outcomes. Understanding these predictive factors may be useful for clinical decision-making and patient management.

2:00-2:50 Session Three

Re-expression of miR-200c/141 in triple-negative breast cancer cells suppresses Notch signalling and impairs tumour growth and metastasis

M. Vaz, K. Watson, K. Simpson, & R. Moorehead* Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph

TNBC, a highly aggressive subtype of breast cancer, tends to have a poor prognosis due to its resistance to targeted therapies. TNBC cells express extremely low levels of the miR-200 family of microRNAs, in particular, the miR-200c/141 cluster. To study the impact of miR-200c/141 re-expression, MDA-MB-231 cells were transfected with either the miR-200c/141 cluster (MDA-231c141) or a control vector (MDA-231EV). Tumours grown by injecting MDA-231c141 cells into the mammary tissue of NCG mice were observed to have significantly lower rates of growth and metastasis as compared to the control, thus suggesting miR-200c/141s involvement in key proliferative and invasion pathways. RNA sequencing revealed that several members of the Notch/Jagged pathway, including JAG1, HES1 and NOTCH2, were significantly downregulated in MDA-231c141 cells and tumours. Quantitative RT-PCR and Western blotting were then used to confirm this trend on the mRNA and protein level, respectively. Immunohistochemistry experiments were subsequently conducted to observe patterns in the quantity and location of JAG1, HES1 and NOTCH2 proteins. The impact of Notch/Jagged signaling on in vitro proliferation and migration of TNBC will also be investigated by inhibiting this pathway in an MDA-231 cell line using both inhibiting drugs and a CRISPR knockout of the JAG1 gene. Analysis of proliferation for this cell line will be conducted using BrdU flow cytometry and 3-dimensional culture. Invasion capability of Notch-inhibited cells will also be evaluated using transwell invasion assays. This study aims to improve our understanding of Notch/Jagged signaling in TNBC and evaluate this pathway as a potential therapeutic target.

Funding: CIHR, University of Guelph

Investigating ABC proteins in triple-negative canine mammary cancer

R.M. Sousa[1], D.R. Pinheiro[2], R.M.R. Burbano[3], B.N. Borges*[1]. 1. Molecular Biology Laboratory, Institute of Biological Sciences, Federal University of Pará, Belém, Brazil; 2. Federal Rural University of Amazonia, Paragominas, Brazil; 3. Molecular Biology Laboratory, Ophir Loyola Hospital, Belém, Brazil

The ATP-binding cassette (ABC) transporter superfamily are transmembrane proteins critical for multidrug resistance phenotype development by controlling cellular efflux pumps of drugs and other substrates. In humans, they are involved in important tumoral metabolic adaptations, like in triple-negative breast cancer (TNBC) progression. However, information regarding the expression patterns of ABC superfamily in dogs with triple-negative mammary cancer (TNMC) subtype remains limited. Considering the above, our study aimed to identify and analyze the expression of ABC proteins in female dogs with TNMC subtype without prior chemotherapy. Mammary tissue

biopsies from five dogs with TNMC, and two control samples were obtained from a Veterinary Hospital in Brazil. The triple-negative subtype was determined by immunohistochemistry. Membrane proteins were isolated and analyzed via Western blot and quantitative proteomics through LC-MS/MS. MASCOT and SwissProt were used to compare the data to the mammalian database. The ABC proteins list was submitted to g:Profiler and EnrichmentMap analysis in Cytoscape. Our study identified 19 distinct ABC family proteins. Gene ontology (GO) analysis highlighted 18 proteins in critical biological processes and pathways (p-value ≤ 0.05), including drug efflux transmembrane transport and long-chain fatty acid transport, as initial expectations. Furthermore, our results suggest their role in established pathways, such as mitochondrial ABC transporters (4/10), ABC transport in lipid homeostasis (6/29), and peroxisomal membrane protein Class I import (3/20). Our findings provide valuable insights into the profile of ABC proteins in TNMC and potentially contribute to therapeutic strategies to modulate this superfamily expression in these patients.

Keywords: Breast cancer; Proteomic; Dogs
Funding: CNPq, CAPES, UFPA

Nck adapter proteins regulate breast cancer cell metastasis and invasion

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The adaptor proteins Nck1 and Nck2 are well established signaling nodes in pathways regulating actin cytoskeleton remodeling. Work from multiple laboratories suggests Nck may be involved in regulating processes correlated with invasion and metastasis of breast cancer, and although these proteins were first identified as oncogenes nearly 30 years ago, there is scarce in vivo evidence supporting their ability to induce tumour development or metastasis. We have now determined that Nck1 and Nck2 are central regulators of breast cancer progression. We have systematically profiled Nck across TCGA-BRCA and related datasets and identified upregulation of Nck in breast cancer which correlates with negative outcomes. We confirmed these findings in patient tumours, and further showed that overexpression of Nck1 and Nck2 in breast cancer cells results in enhanced invasion and gelatin degradation. Next, using an in vivo loss of function strategy in mice which allows simultaneous expression of activated oncogene HER2/ErbB2 and Cre recombinase in mammary epithelial cells, we have shown that deletion of both Nck1 and Nck2 (Nck-DKO) significantly extends survival by delaying tumour onset and also reduces incidence of metastasis. Protein analysis of tumours lacking Nck1 and Nck2 shows significant alterations to focal adhesion signaling dynamics. To identify key Nck-dependent regulators, we have used CRISPR to generate matching WT and Nck-DKO cell lines and performed RNA-seq. Our findings provide new

physiological insights verifying the role of Nck as an oncogene, and they reveal its potential as a target to inhibit breast cancer.

Identification of lysyl oxidase as an adipocyte-derived inducer of morphological changes in MDA-MB-231 cells

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Breast cancer (BC) is the most common and the second deadliest cancer among women worldwide. Multiple meta-analyses have clearly identified obesity as a risk factor for BC incidence and overall survival where obesity is associated with a 15-50% increased risk depending on menopausal status, BC subtype, and method of evaluating obesity. The mechanisms underlying this increased risk are not fully understood. Previously, we have shown that adipocytes promote the shift of triple-negative breast cancer (TNBC) cells from a spread, mesenchymal phenotype to a rounded, luminal/epithelial-like phenotype when cells are grown in 3-dimensional (3D) culture. Critically, our data showed that adipocytes are only able to cause this mesenchymal to epithelial transition (MET) in TNBC cells, when they are cultured in the presence of extracellular matrix (ECM) provided as laminin-rich Matrigel. To identify adipocyte-derived mediators of this partial MET, we conducted liquid chromatography tandem mass spectrometry of conditioned media (CM) of adipocytes cultured in the presence (AE-CM) or absence (A-CM) of overlaid Matrigel. We identified 91 proteins that were exclusively present in the AE-CM. Assessment of published datasets of breast cancer patients identified associations of 6 of these proteins with overall patient survival. We further investigated the capacity of mature adipocytes to express the mRNA (qRT-PCR) or secrete (ELISA) four of these proteins in the absence or presence of overlaid Matrigel, and in two different culture media. Based on protein secretion data, we further tested lysyl oxidase (LOX) for its capacity to promote MET-like morphological changes of MDA-MB-231, a human TNBC cell line, in both 3D and 2D cultures on Matrigel. E-cadherin, ZO-1, and vimentin expression were assessed by immunoblotting following LOX exposure. Our findings suggest that LOX is one of the adipocyte-derived mediators of partial MET in TNBC cells and should be further investigated in the context of obesity-associated BC.
Funding: NSERC, Cancer Research Society, Art Rouse Memorial Scholarship from the Ontario Veterinary College.

POSTER ABSTRACTS

Posters will be available for viewing all day; questions can be emailed to the authors as indicated on the poster page. There will be no live interactive poster session other than those being presented during the short talks. There will be no judging for the poster presentations this year. The posters can be viewed at https://icci.uoguelph.ca/icci-annual-symposia/2023-icci-virtual-cancer-research-symposium/2023-icci-symposium-posters/.

Creating accessible microarray platform for detection of cancer-related proteins

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Introduction: Microarrays can be used for powerful high-throughput multiplexed bioassays for cancer research and management; however, their use has been limited as their fabrication requires a costly and complex microarray printer unavailable to most labs. Furthermore, mixing different bioreagents in a multiplexed assay leads to cross-reactions, producing false positive signals which impair assay reproducibility and scalability.

Methods: In this work, we propose a new microarray format, named Compartmentalized Linker Array (CLA) that consists of pre-prepared storable microarrays of chemical linkers in microliter compartments. CLA can be used for binding and patterning bioreagents into microarrays via simply pipetting and incubating microliters of bioreagent solutions in the compartments, instead of printing nanoliters of bioreagent.

Results: Using an aminosilane linker-based antibody microarray, we developed CLA and demonstrated its application for a multiplexed sandwich immunoassay measuring three cancer-related proteins. The CLA's limits of detection for EGFR, TNF-α and GM-CSF are 2.5 pg/mL, 3.5 pg/mL and 2.2 pg/mL, respectively. These are comparable to ELISA which detects these proteins at 36 pg/mL, 6.2 pg/mL, and 3.0 pg/mL respectively. Additionally, the CLA only uses 2 µL of each reagent and sample to fill a compartment, approximately 50 folds lower than those required in conventional assays.

Conclusions: CLA can potentially transcend the antagonism among multiplexing capability, reliability, and cost of multiplexed bioassays that has prevented their broader adoption in cancer research. Future work will demonstrate that the CLA is not limited to protein measurement but can provide versatile tools for a wide range of applications, such as those for the studies of cells and extracellular vesicles.

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Disclosures: A patent has been filed on the proposed compartmentalized linker array technology, with H.L. and R. A. as the inventors.

The Impact of miR-200s in high grade serous ovarian cancer

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Epithelial ovarian cancer (EOC) is the fifth leading cause of cancer-related deaths in women and is the most lethal gynaecological cancer. High-grade serous ovarian cancer (HGSOC) predominates in the clinical setting and is commonly diagnosed at late stages accompanied by peritoneal metastases. It may prove beneficial then to study how to alter the phenotype of EOC cells to decrease their invasive characteristics. The miR-200 family of microRNAs works to inhibit the epithelial to mesenchymal transition (EMT), which is critical for cancer progression. This family consists of two clusters, miR-200c/141 and miR-200b/200a/429. By over-expressing one or more miR-200 family clusters in HGSOC cells, an epithelial phenotype may predominate to suppress cancer growth and metastasis. To test this hypothesis, either or both clusters of the miR-200 family will be lentivirally expressed in a highly aggressive HGSOC cell line, 28-2. Expression of EMT-related genes will be tested, with the hypothesis that they will be suppressed to revert the EOC cells to a more epithelial phenotype. Then proliferation and apoptosis will be measured. For the in vivo experiments, 28-2 control and miR-200 overexpressing cells will be injected into the ovarian bursa of syngeneic C57BL/6 mice. 60 days post injection, ovarian tumors will be removed, measured, and weighed. In addition, the number of peritoneal metastases will be counted. The collected ovaries will be used to determine tumour cell proliferation and angiogenesis. Overexpression of the miR-200 family in a highly aggressive model of HGSOC may be sufficient to alter cell morphology, impair metastasis and decrease proliferation.

Funding: Ontario Veterinary College, CIHR

Investigation of the effects of a Wee1 inhibitor, MK-1775, in combination with carboplatin or doxorubicin on canine splenic hemangiosarcoma cells

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Hemangiosarcoma is an aggressive malignant neoplastic disease that accounts for 45-51% of all splenic malignancies and is the leading cause of nontraumatic hemoabdomen in dogs. Its human equivalent, angiosarcoma, is a rare and poorly understood cancer with no optimal treatment, due to small cohort studies with incomplete data and varying treatment approaches, all of which indicate poor survival rates. Hence, there is an urgent need for new therapeutic strategies for these cancers. The standard-of-care treatment for canine splenic hemangiosarcoma (csHSA) includes a splenectomy followed by doxorubicin, resulting in a median survival of 4-7 months and a 1-year survival rate of <10%. A recent retrospective study suggests that carboplatin has a similar efficacy for treating csHSA when compared to doxorubicin with more acceptable clinical tolerability. However, as response rates to chemotherapy remain low, chemosensitizers have been of interest. Inhibition of Wee1, a critical kinase that mediates G2/M cell cycle arrest, may cause csHSA cells

to enter mitosis despite DNA damage from chemotherapy, leading to mitotic catastrophe. We hypothesized that there is synergism between doxorubicin or carboplatin and MK-1775 (Wee1 inhibitor), and that combination drug treatment would increase cell death by preventing DNA damage repair in cancer cells preferentially. A csHSA cell line (DD-1) was treated in vitro with doxorubicin and carboplatin as sole treatments or combined with MK-1775. Cell viability and drug interaction were evaluated by crystal violet assay and combination index (CI) calculation, respectively. When combined with MK-1775, doxorubicin or carboplatin exhibited strong synergism (CI<<1) in reducing DD-1 cell viability. Further evaluation of apoptosis, DNA damage, and target modulation are underway using immunoblotting. Keywords: Hemangiosarcoma, Angiosarcoma, Comparative Oncology, Doxorubicin, Carboplatin, MK-1775, Wee1

The Impact of miR-200s on HER2+ breast cancer cell migration and proliferation in vitro and tumour growth and initiation in vivo

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Human Epidermal Growth Factor Receptor 2 positive (HER2+) breast cancer is diagnosed in 20% of all breast cancer patients and patients with HER2+ breast cancer have poor prognoses. While this form of breast cancer is treatable, the survival rate has only slightly improved as resistance frequently occurs. The miR-200s family has been characterized by our lab previously to inhibit tumour initiation, reduce tumour growth, reduce metastasis and proliferation in triple negative breast cancer but the impact of miR-200s in HER2+ breast cancer remains largely unexplored. This study used two HER2+ breast cancer cell lines and transgenic mice to investigate the impact of miR-200 overexpression on proliferation, apoptosis and migration in vitro and tumour growth in vivo. Overexpression of the miR-200ba429 cluster in HER2+ breast cancer led to significant reductions in migration rate, proliferation while also significantly increasing the rate of apoptosis in both cell lines. The in vivo overexpression of the miR-200ba429 cluster prevented tumor initiation induced by Neu (rodent version of HER2). Together, the in vitro and in vivo research shows that the miR-200 family negatively regulates tumor initiation, proliferation and migration of HER2+ breast cancer.

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Exploring the ability of Fc3TSR to enhance the efficacy of immunotherapies, including checkpoint inhibitors and chimeric antigen receptor T-cell therapy, in the treatment of ovarian cancer

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Epithelial Ovarian Cancer (EOC) is typically diagnosed at advanced stages and has a poor prognosis despite recent advancements. Ovarian tumours are characterized by rapid onset of chemoresistance, irregular vasculature, and altered lymphatic vessel morphology leading to elevated interstitial fluid pressure, tissue hypoxia, and poor tissue perfusion. This research investigates the ability of Fc3TSR to improve the treatment of EOC by inducing direct effects to resensitize chemoresistant tumour cells and remodel the tumour microenvironment to enhance the efficacy of combination therapies. To investigate the role of Fc3TSR in chemoresistance, in vitro cell survival will be assessed after treatment with varying doses of carboplatin with or without 10nM Fc3TSR. In vivo, mice implanted with ID8-R cells will be treated with different combinations of PBS, Fc3TSR, and metronomic chemotherapy. To determine whether Fc3TSR can enhance the uptake of immune checkpoint inhibitors (ICIs), monoclonal antibodies targeting PD-1 and CTLA-4 will be conjugated to fluorochromes and administered as clinically relevant monotherapies or combined with ICI + Fc3TSR in orthoptic synergistic EOC models. To characterize the anti-tumour effects of combined Fc3TSR and immune cell therapies, Fc3TSR will be used to enhance the delivery of adoptively transferred CD8+ effector cells into the tumour microenvironment. Results demonstrate that Fc3TSR reduced interstitial pressure, normalized tumour lymphatic vasculature, and enhanced migration of immune cells to the draining lymph nodes in an advanced-stage EOC mouse model. The findings suggest that Fc3TSR can improve the efficacy of cancer therapeutics by decreasing the tumour's immunosuppressive environment and increasing the migration of immune cells.
Funding: CIHR, Ovarian Cancer Canada

Effect of volume on fluorescence intensity and transit of indocyanine green for sentinel lymph node mapping in a simulated feline tumour model

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Sentinel lymph node (SLN) mapping via a combination of preoperative and intraoperative techniques allows for a better understanding of the primary tumour drainage pathway(s). Intraoperative methods include the use of near-infrared fluorescence imaging (NIRF) and the injections of dyes such as indocyanine green (ICG) and methylene blue (MB). Though frequently used in human oncology, the use of SLN mapping with ICG and MB has not been widely adopted in veterinary medicine.

The objectives of this study were to compare the effect of volume and solution of percutaneous fluorescence intensity (FI) and transit time in a simulated tumour model in cats. Seven clinically healthy, 1 year old male intact purpose-bred cats were randomly divided into three groups. Both pelvic limbs were clipped and the assigned solution (either solution A (1mL) or solution B (2mL) of ICG +/- MB) was injected intradermally in 4 quadrants around the simulated tumour on the

dorsal metatarsus. Under NIRF imaging, the transit time to the SLN (ipsilateral popliteal LN) and the FI at the injection site, lymphatic tract and SLN were recorded.
The overall transit time to the SLN was decreased with a significantly faster velocity ((p = 0.001; range 0.5 – 3.42 minutes) when a larger volume of dye was injected. The overall FI was not affected by the volume of dye, type of dye or when massage was applied. No adverse reactions were reported in all 7 cats up to 1 month following the procedure, therefore SLN mapping with ICG +/- MB should be considered in cats with neoplastic disease.
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Evaluation of canine mast cell tumour sensitivity to oncolytic viruses

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Canine cutaneous and subcutaneous mast cell tumors [MCTs] are highly prevalent. There are many standard of care treatments for pet cancer, but many are not effective. In human cancer therapy, interest in oncolytic virotherapy is a rapidly growing.
Canine neoplastic cells can be treated with oncolytic viruses (OV), which can selectively kill tumor cells and stimulate an anti-tumor immune response. This study evaluated different OVs for their therapeutic potential against a dermal canine MCT, which was isolated from a 7-year-old male castrated Sharpei dog (MCT-1). The OVs tested included recombinant vesicular stomatitis virus (rVSV Δm51), Newcastle disease virus (NDV), and Parapoxvirus or Orf virus (ORFV), all of which were green fluorescent protein (GFP) tagged.
The results of our study showed that MCT-1 cells infected with different OVs had varying levels of GFP expression in the flow cytometry data. The effectiveness of the OVs was also measured through an in vitro resazurin dye-based metabolic assay, which demonstrated that rVSV Δm51 was taken up the most by MCT-1 cells, of all the viruses tested. These findings suggest that rVSV Δm51could be a safe and effective treatment for MCT-1 cells in the future.
Keywords: Cancer, MCT, Oncolytic Virus.
Funding: OVC Pet Trust

A pilot evaluation of the dose- and YAP-dependent effects of verteporfin in canine and mouse osteosarcoma cells

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Osteosarcoma (OS) is the most common bone cancer in dogs. Canine OS treatment involves surgery and chemotherapy; however, the majority of patients will succumb to metastatic disease within 1 year. Unfortunately, clinical trials have failed to provide significant benefits for patients, highlighting the need for novel therapies. Photodynamic therapy (PDT) is a treatment modality that employs light to activate a photosensitizer, which in turn generates reactive oxygen species to cause tumour cell death. We believe that PDT employing the photosensitizer verteporfin (VP) holds promise for the treatment of canine OS. VP-PDT is approved for the treatment of neovascular eye disease in humans, and both VP-PDT and VP alone were shown to inhibit tumour growth in mouse models of cancer, including OS. The anti-tumour activity of photoinactive VP has been attributed to its interference with Hippo signalling, specifically through the targeting of yes-associated protein (YAP). Similar to other photosensitizers, VP has also been observed to induce immunogenic cell death (ICD) when used in PDT, which might be beneficial to suppress metastasis.

We hypothesize that VP will cause ICD of OS cells in a YAP-targeting and dose-dependent manner, with maximum effects following photo-activation. To demonstrate this, we treated metastasis-derived canine (D17 and OVC-cOSA-31) and mouse (K7M2) OS cell lines with increasing doses of VP without light for 24 hours, and then assessed the effects on YAP levels by immunoblotting. Next, we treated cells with YAP-targeting and minimally-targeting doses, both with and without activating light (LED, 635 nm). Cell viability, apoptosis, and ICD were assessed using a WST-1 assay, immunoblotting and immunofluorescence. The results indicate that a YAP-targeting dose of VP is more effective at reducing viability of canine OS cells and at promoting ICD when activated by light. Our results suggest that VP-PDT could be optimized for the treatment of canine osteosarcoma.

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Tumour suppressor gene copy number in whales

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Low cancer incidence rates are observed in cetaceans, despite their large mass and long lifespan hypothetically contributing to a higher lifetime probability of acquiring oncogenic mutations. Few studies have investigated cancer-related genes in cetaceans. One reported that bowhead whales possess two functional copies of PCNA and suggested that the extra copy could contribute to their cancer resistance due to the gene's role in DNA repair. Droplet digital PCR (ddPCR) was used to quantify gene copy number for TP53, PCNA, HER2, DLG1, and DLG2 in genomic DNA extracted from frozen skin samples of belugas, narwhals and bowheads (n=20 each). Results showed that all 3 whale species had more than one copy of PCNA. Traditional PCR showed the simultaneous

presence of wild-type PCNA (possessing introns) and variable numbers of pseudogene sequences (lacking introns) within individuals for belugas, narwhals, and bowheads. To investigate copy number loss in cancer, ddPCR was also performed on formalin-fixed, paraffin embedded normal and matched tumour tissue of 7 individual belugas from the St. Lawrence estuary, an area that was historically contaminated with industrial carcinogens. Copy number loss was not observed for any investigated TSG in tumour tissue compared to normal tissue. Our results show that bowhead whales are not unique in having multiple copies of PCNA. Further study is required to understand the significance of the variable PNCA pseudogene copies. Understanding natural copy number variation in TSGs may provide insight into risk factors and prevention methods across species.
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In vitro evaluation of optimal indocyanine green preparation methods for near-infrared imaging

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Indocyanine green (ICG) is a near-infrared (NIR) fluorophore used in various clinical applications1–3. ICG is used for sentinel lymph node mapping (SLN) during surgery for detection of possible metastatic nodes2. A challenge with using ICG is the occurrence of quenching which decreases fluorescence intensity and is influenced by dilution methods. To improve clinical efficacy and reproducibility in SLN mapping research, there is a need to understand and optimize the dye itself. There is a lack of consensus regarding the optimal ICG preparation method for local injection1,4.

Four canine melanoma cell lines (CML1, CML6M, CML10C2, 17CM98) were be seeded on coverslips and 96-well plates, to replicate a tissue bed in vitro. The cells were incubated at various concentrations that were diluted in water, saline, dextrose, and albumin. For microscopy, the cells were labelled with an anti-E Cadherin antibody and mounted with DAPI onto slides to be imaged with a confocal microscope. The 96-well plates were imaged with the Odyssey DLx. For quantitative analysis, the mean fluorescence intensity was calculated using ImageJ software and Empiria Studio. This experiment was done in triplicate.

Cell lines incubated with ICG diluted in water, saline, and dextrose had strong MFI compared to other cell lines and preparations, such as diluting with albumin ($p < 0.05$). ICG preparations with isotonic solutions result in cell viability post-ICG treatment compared to ICG diluted with water. Analysis histograms depict that cells treated with ICG albumin show ICG localized in the membrane and cytoplasm, whereas ICG in dextrose and saline localize in the nucleus and cytoplasm.

Funding: OVC Pet Trust

The EphB4 receptor regulates canine and human osteosarcoma invasiveness and tumorsphere formation

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Osteosarcoma is a highly aggressive bone cancer in both canines and humans with a high rate of metastasis and corresponding poor prognosis. Advances in treatment options are limited, highlighting the need for more effective therapeutic approaches.

The Eph receptors are the largest group of receptor tyrosine kinases, regulating many cellular activities including proliferation, survival, migration, and invasion. Effects of altered expression of Eph receptors on tumor aggressiveness have been characterized in multiple human malignancies, making these receptors attractive targets for therapeutic intervention. Recent evidence suggests that the EphB4 receptor is involved in the regulation of invasion and metastasis of various human cancers. However, the role of the EphB4 receptor in osteosarcoma has been poorly evaluated. Due to the physiological and cellular similarity between canine and human osteosarcoma, we are using a comparative approach to investigate the role of the EphB4 receptor in promoting osteosarcoma.

We found upregulated expression of the EphB4 receptor in canine and human osteosarcoma cells when compared to normal osteoblasts. Our initial experiments demonstrated that the knockdown of EphB4 corresponded with reduced cell proliferation. Subsequently, we investigated the effects of EphB4 on osteosarcoma cell migration and invasion and on propagation of tumor-initiating cells (TICs). Interestingly, EphB4 knockdown reduced cell migration and invasion and enhanced TIC proliferation in both canine and human osteosarcoma. This demonstrates that the EphB4 receptor regulates important processes in the development and invasiveness of osteosarcoma. Given that similar results were observed in both species, this emphasizes the benefit of using a comparative oncological approach.

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ShcD adaptor protein modulates EGFR signalling and invasion in breast cancer cells

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Triple-negative breast cancers are highly metastatic and present clinical challenges as there are currently no effective therapies. While metastasis is the leading cause of breast cancer mortality, the underlying molecular mechanisms are unclear, and identification of new regulators is crucial.

The ShcD phosphotyrosine adaptor protein bridges signalling complexes to classes of receptor tyrosine kinases implicated in metastatic signalling pathways. ShcD shares similar structure with paralog ShcA, which has an established role in mammary tumorigenesis and progression. Here we have identified ShcD upregulation in triple-negative tumours which correlates with overall reduced patient survival. We show that in human breast cancer cells, ShcD expression significantly enhances ligand-stimulated EGFR phosphorylation, reduces cell adhesion, and heightens cell invasion in vitro, with opposing effects upon ShcD knockdown. Furthermore, in a three-dimensional system, we report that ShcD expression enhances the infiltration of spheroids derived from a brain metastatic breast cancer cell line into human cerebral organoids. In each event, effects are mitigated with a ShcD mutant that can no longer engage surface receptors like EGFR or signal to downstream pathways involving Gab1 and Akt. Lastly, we show that treatment of breast cancer cells expressing ShcD with anti-inflammatory drug indomethacin decreases associations between ShcD and EGFR and reduces EGFR phosphorylation, which correlates with reduced cell invasion. Our results link ShcD-induced EGFR hyperphosphorylation to the modulation of metastatic properties and position ShcD as a putative contributor to breast cancer progression. Moreover, we provide a molecular basis for clinical targeting of adaptor-RTK interactions in breast cancers.
Funding: Cancer Research Society

Influence of storage conditions on indocyanine green stability and fluorescence intensity

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Indocyanine green (ICG) is a near-infrared fluorescent dye used in several medical applications including sentinel lymph node (SLN) mapping for cancer patients. As metastasis typically occurs via the lymphatics, failure to understand the spread of a primary tumour and identification of SLNs lead to incomplete treatment, worse prognosis, and poor patient outcome.

Indocyanine green is a favourable dye due to its safety profile, short lifetime in blood circulation, good signal-to-noise ratio, and imaging capabilities. Prior to use, ICG powder must be mixed with sterile water. Once reconstituted the dye begins to degrade, as such the manufacturer's recommendation is to use within 6 hours to ensure quality of fluorescence intensity (FI). In veterinary SLN mapping, small quantities of ICG (0.1mL or 1/10th of a bottle) are used which results in waste due to its short shelf life and limits accessibility due to the high bottle cost (~$450 CAD).

To develop validated clinical protocols for storage and use, the stability and FI of ICG after extended storage in 4◦C, 20-25◦C, -20◦C and -80◦C must be explored. Human ovarian epithelial cancer cells (Coav-3) and normal human ovarian surface epithelial as a control, were treated with ICG and evaluated at 1, 12, 24, 36, 48, 60, 72, 84, 96, 108, 120 hours and 1, 2, 3, 4, 6, 8, 14 and 29 weeks. Evaluation of FI was completed using the Odyssey CLx imager via a black 96-well microplate with data analyzed by ANOVA.

Preliminary results suggest that when stored at room temperature, there are subjective changes in the colour of the solution and following 5 days, there is no perceivable fluorescence in the NIRF window (650-775nm). Interestingly, when stored in the freezer (-20 and -80◦C) ICG has a similar FI to the initial preparation.
Funding: OVC Pet Trust

The Institute for Comparative Cancer Investigation Companion Animal Tumour Sample Bank: facilitating translational cancer research

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The Companion Animal Tumour Sample Bank (CATSB) facilitates basic and translational veterinary oncology research. Located in the OVC Mona Campbell Animal Cancer Centre, the CATSB is the only veterinary oncology biobank in Canada and is registered with two national repository networks. With a current repository of over 1,850 cases, samples collected and stored include serum, plasma, buffy coat, urine, and tissue. Normal tissue and tumour samples are collected immediately following surgical excision and are available as flash frozen and RNAlater- and CryoMatrix-preserved. Tumour tissue is also formalin fixed and analyzed by a pathologist for quality control. In addition to standard preparations, prospective sampling can be adapted for specific projects. A wide variety of neoplasms have been collected, the most prevalent of which in dogs are soft tissue sarcoma (STS), lymphoma and osteosarcoma; and in cats, STS, mammary carcinoma and osteosarcoma. There are also 11 cell lines from primary tumours, with more in development. The CATSB collects all case-related data for patients with banked samples facilitating retrospective analysis. The process to request samples is straightforward: How to Request Samples (ICCI website). Samples and data from the CATSB have been used in 32 intramural and extramural research projects to date. The CATSB is a unique resource with the mission to facilitate basic, comparative, and translational research to improve the lives of companion animals with cancer. In addition, data from spontaneous companion animal tumours can complement preclinical rodent studies, with the augmented potential to contribute to comparative human cancer research.
Funding: OVC Pet Trust and The Smiling Blue Skies Cancer Fund

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Analysis of oxidative stress on off-target infection of activated T cells by oncolytic vesicular stomatitis virus

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The rapidly progressing field of oncolytic virotherapy takes advantage of the ability of genetically modified viruses to selectively infect and kill cancer cells and induce tumour-specific immune responses. Oncolytic viruses (OVs), such as vesicular stomatitis virus (VSV), preferentially target cancerous cells due to various mechanisms such as the utilization of the defective antiviral defenses of tumour cells. However, success has been limited partially due to the ability of some OVs to cause off-target infection of leukocytes including activated T cells, reducing therapeutic potential. This could be problematic for multi-dosing protocols in which the first dose of VSV activates T cells that can then be infected with subsequent doses of the virus. Previous studies have demonstrated that antioxidants can prevent viral infection due to their ability to reduce concentrations of reactive oxygen species. This study aimed to determine whether antioxidants could prevent infection of T cells by VSV. Splenocytes from C57BL/6 mice were exposed to antioxidants in vitro and flow cytometry was used to ascertain the T cell infection with VSV expressing a fluorescent protein. Treatment with N-acetyl-L-cysteine (NAC) or catalase decreased the frequency of infection with VSV. Importantly, neither antioxidant affected the viability of murine B16F10 melanoma cells or human HeLa cervical cancer cells, demonstrating that while NAC and catalase may prevent the infection of T cells, they do not prevent the infection and killing of tumour cells. These results could improve cancer immunotherapies by decreasing off-target infection of T cells when paired with antioxidants already approved for clinical use.
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Immunogenic cell death in triple negative mammary cancer cells treated with 5-aminolevulinic acid photodynamic therapy

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Breast cancer (BC) is the primary cause of death in women worldwide. Triple negative breast cancer (TNBC) is challenging due to its highly diverse, multidrug-resistant, and immunosuppressive nature. As no effective treatment is available beyond cytotoxic chemotherapy, it is crucial to explore effective strategies to induce an immune system's antitumour response. Five-aminolevulinic acid (5-ALA) photodynamic therapy (PDT) is a non-invasive treatment approach that utilizes selective accumulation of photosensitizer Protoporphirin IX (PpIX) in cancer cells; when exposed directly to red light, PpIX is converted to 5-ALA producing cytotoxicity by generating reactive oxygen species and the release of damage-associated molecular patterns (DAMPs) that stimulate immunogenic cell death (ICD) in tumor cells without damaging the surrounding tissue. The purpose of this study was to characterize the exposure of immunogenic treats by TN mammary cancer cells exposed to 5-ALA PDT. We hypothesized that 5-ALA PDT-treated TN mammary cancer cells will expose ICD biomarkers

such as HMGB1, calreticulin, and HSP90B1 along with apoptosis biomarkers (CC3, CC8 or CC9) and/or pyroptosis biomarkers (CC1, Gasdermin D N-terminal fragment) that confirm cell death type. Therefore, we treated monolayers of murine TN mammary cancer cell line 4T1 and its human equivalent MDA-MB-231, as well as 2 non-transformed murine mammary cell lines (NMuMG and EPH4) with 2 concentrations of 5-ALA (40 and 80 □g/mL) and applied red light of 635 nm at a dose of 50 J/cm2 (fluence rate of 100 mW/cm2) for 12 minutes, using a LED source. Cell viability, apoptosis and ICD were assessed using a crystal violet assay, immunoblotting and immunofluorescence respectively. Biomarkers expression indicates that both 5-ALA concentrations plus the harnessed light cause cell death in all cell lines suggesting the need of light dose optimization to target tumour cells selectively. The expression of ICD biomarkers confirms the potential of PDT to be immunogenic.
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Differential regulation of the KEAP1-NRF2 antioxidant pathway in long and short-lived bird species

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Oxidative stress increases the activity of the transcription factor NRF2 which induces the expression of hundreds of diverse antioxidant genes. NRF2 regulation occurs mainly through KEAP1 which constitutively targets NRF2 for degradation unless electrophiles or oxidative stressors alter the structure of KEAP1, preventing it from binding NRF2. Intracellular levels of KEAP1, but not other protein-level attenuators of NRF2, are negatively associated with lifespan across various species. Excessive NRF2 activity promotes hypoxic survival, angiogenesis, chemoresistance, and metabolic shifts favouring tumour progression. Human lung adenocarcinomas, renal carcinomas, and squamous cell carcinomas commonly harbour loss-of-function mutations affecting KEAP1 and NRF2 binding residues, but the consequences are poorly understood compared to mutations in other cancer-associated genes. Insight into NRF2's role in cancer might be gained by studying Neoaves (e.g., parrots), a clade of birds whose KEAP1 lacks the NRF2 binding region due to truncation. Neoaves do not display accelerated tumour progression as would be expected from aberrant NRF2 activity but instead have much longer lifespan compared to basal aves species (e.g., chickens) and similarly sized mammals. These combined observations suggest that KEAP1-independent regulators are important for controlling Neoavian NRF2 activity. To identify protein-level NRF2 interactors in Neoaves, protein lysates were extracted from liver tissue of Neoaves and basal aves species followed by coimmunoprecipitation of NRF2-bound proteins and SDS-PAGE. The bands representing NRF2-specific protein interactors indicated by SDS-PAGE were examined through mass spectrometry, identifying potential NRF2-binding proteins. Future immunohistochemistry work will examine the expression of NRF2-binding proteins using avian neoplasia tissue microarrays.

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A novel benzophenanthridine alkaloid, 6ME, targets acute myeloid leukemia (AML) involving peroxisome proliferator-activated receptors (PPARs)

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Acute myeloid leukemia (AML) is a devastating hematological malignancy characterized by uncontrolled proliferation and accumulation of undifferentiated myeloid precursors (myeloblasts) in the peripheral blood, bone marrow, and/or other tissue leading to impaired hematopoiesis and bone marrow failure.

Existing treatment strategies for AML therapy (e.g., chemotherapy) can induce remission but are associated with relapse while also posing serious adverse effects on the healthy/normal cells and individual's health. Therefore, alternatives are needed to improve patient outcomes. A high throughput flow-cytometry based screen, in multiple leukemia cell lines, identified a novel benzophenanthridine alkaloid, 6ME, as a novel candidate with anti-AML potential. Our preliminary results show 6ME exhibits impressive and selective cytotoxicity against AML cells (i.e., cell lines and patient-derived; IC50 values around 0.8-1μM) by the 7-aminoactinomycin (7-AAD) assay and clonogenic growth assay with patientderived AML cells and normal peripheral blood stem cells (PBSC) from healthy donors. Moreover, published computational methods were employed to predict potential targets of 6ME and identified peroxisome proliferator-activated receptors (PPARs) as the potential target. Meanwhile, coimmunoprecipitation (coIP) and immunoblotting were also conducted to study the role of PPARs in 6ME's anti-AML activity, which indicated 6ME lowered the expression of PPARs. Taken together, we hypothesize 6ME selectively targets AML involving PPARs, and thus this project aims to elucidate the molecular mechanisms of 6ME-induced AML cell death, with a specific focus on its regulation of PPARs-mediated pathways.
