UNIVERSITY of GUELPH

INSTITUTE FOR COMPARATIVE CANCER INVESTIGATION

# 14th Annual ICCI

# Cancer Research Symposium

## Thursday May 12, 2022

## Pheedloop Web Platform 9:00-4:00

# Introductory Remarks

Welcome to the 14th 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. Elizabeth Murchison is the 2022 Arthur Willis Distinguished speaker and will be giving the keynote address at 9:05 a.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

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**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, Pheedloop Webinar Services for help with the virtual meeting platform, and Dr. Vicky Sabine for help with abstract reviews and organization.

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, Animal Health Partners Research Chair in Veterinary Medical Innovation, Art Rouse Memorial Scholarship, Canadian Institutes of Health Research, Cancer Research Society, Companion Animal Health Fund, Memorial University of Newfoundland, Natural Sciences and Engineering Research Council of Canada, Ontario Institute of Cancer Research, Ontario Ministry of Agriculture, Food & Rural Affairs, OVC Pet Trust, Saskatchewan Health Research Foundation, Terry Fox Research Institute, The Smiling Blue Skies Cancer Fund and University Health Network.

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# ICCI 14th Annual Cancer Research Symposium, Thursday May 12th, 2022

## Morning Session

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

**9:05-10:15 Keynote Speaker**

*Transmissible cancers: when cancer cells become infectious agents*

**Dr. Elizabeth Murchison**; Transmissible Cancer Group, Department of Veterinary Medicine, University of Cambridge

10:15-10:55 Guest Speaker

*Applying deep learning to tumour pathology*

**Dr. Christof Bertram**; Department of Pathobiology, University of Veterinary Medicine, Vienna

10:55-11:15 1st round poster presentations

11:15-11:50 Short talks from abstracts. Moderator: Michelle Oblak

1. *microRNA expression in canine appendicular and metastatic osteosarcoma cell lines and tissues*
   **Dr. Latasha Ludwig;** Department of Pathobiology, University of Guelph

2. *ShcD adaptor protein modulates EGFT signaling and invasion in breast cancer cells*
   **Dr. Hayley Lau;** Department of Molecular and Cellular Biology, University of Guelph

3. *Assessing the sexual health needs of patients with prostate cancer accessing a regional cancer centre*
   **Dr. Tuuli Kukkonen;** Associate Professor, Family Relations & Applied Nutrition, University of Guelph

11:50-12:30 Guest Speaker

*Cancer Immunotherapy Challenges and Opportunities: Perspectives from a "Battle-Hardened" Immunologist*

**Dr. Laszlo Radvanyi**; President and Scientific Director, Ontario Institute for Cancer Research

**12:30-1:15** ***Poster Viewing and Lunch Break***

## Afternoon Session

1:15-1:35 2nd round poster presentations

1:35-2:10 Short talks from abstracts and invited speaker. Moderator: Alicia Viloria-Petit

4. *High Intensity Focused Utrasound (HIFU)*
   **Dr. Brigitte Brisson;** Professor of Small Animal Surgery, Department of Clinical Studies, Ontario Veterinary College, University of Guelph

5. *Proteomic profiling of extracellular vesicles from tumour explants as a pipeline for the discovery of biomarkers and targets in canine osteosarcoma*
   **Dr. Alicia Viloria-Petit;** Associate Professor, DOGBONe Co-Leader, University of Guelph

6. *The role of Hippo signalling in OS progression*
   **Anita Luu;** Department of Biomedical Sciences, University of Guelph

2:10-2:50 Short talks from abstracts and invited speakers. Moderator: Alicia Viloria-Petit

7. *A new highly sensitive assay for multiplexed quantification of cancer exosomal proteins in complex biofluids*
   **Dr. Huiyan Li;** Assistant Professor – Biomedical Engineering, University of Guelph

8. *Modeling osteosarcoma metastasis with PDX-derived cell lines*
   **Dr. Courtney Schott;** Assistant Professor – Anatomic Pathology, University of Guelph

9. *Circulating serum cytokine concentrations of IL-8 and IP-10 have potential prognostic value in canine osteosarcoma*
   **Natalie Hillis;** Department of Biomedical Sciences, University of Guelph

2:50-3:10 3rd round poster presentation

3:10-3:55 Guest Speaker

*The CANcer Detection in Dogs (CANDiD) study: A multi-site, international study to develop a noninvasive "liquid biopsy" test to detect cancer with just a blood draw*
**Dr. Andi Flory, DVM, DACVIM (Oncology)**; Chief Medical Officer, PetDx
**Lisa M. McLennan, SCRA**; Director, Clinical Studies & Biobanking, PetDx

3:55-4:00 Closing Remarks

**KEYNOTE PRESENTATION**

**9:05 – 10:15 a.m.**

# Dr. Elizabeth Murchison, PhD

Professor of Comparative Oncology and Genetics at University of Cambridge, Department of Veterinary Medicine

## Transmissible cancers: when cancer cells become infectious agents

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Elizabeth Murchison is Professor of Comparative Oncology and Genetics at the University of Cambridge, Department of Veterinary Medicine. Her laboratory, the Transmissible Cancer Group, studies the genetics, evolution and host interactions of clonally transmissible cancers in dogs and Tasmanian devils. Elizabeth grew up in Tasmania, Australia's rugged southern island state. She obtained her undergraduate degree in genetics and biochemistry in 2002 from the University of Melbourne, and performed doctoral research at Cold Spring Harbor Laboratory, New York, from 2002 to 2007. After a postdoctoral fellowship at the Wellcome Sanger Institute, where she sequenced the genome of the Tasmanian devil and its first transmissible cancer, she joined the University of Cambridge faculty in 2013. She has been the recipient of several awards, notably the Cancer Research UK Future Leaders in Cancer Research Award (2014). Elizabeth is a keen science communicator, and in 2011 she delivered a TED talk entitled "Fighting a Contagious Cancer", which has been translated into 29 languages and viewed by a global audience more than 500,000 times.

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**Past ICCI Symposium Arthur Willis Distinguished Speakers**

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

## GUEST SPEAKER:

### 10:15-10:55

**Applying deep learning to tumour pathology**

Dr. Christof Bertram, Dr. med. vet., PhD; Department of Pathobiology, University of Veterinary Medicine, Vienna

Dr. Bertram is an anatomic pathologist at the University of Veterinary Medicine in Vienna, Austria. The Vetmeduni Vienna is the only veterinary academic educational and research institution in Austria and is the oldest veterinary university in the German-speaking region of Europe. Dr. Bertram graduated from the Department of Veterinary Medicine at the Freie Universität Berlin, Germany, and stayed in Berlin for his doctoral studies and residency at the Institute of Veterinary Pathology. His current duties as a senior lecturer at the Institute of Pathology at the Vetmeduni Vienna are teaching, directing the biopsy and necropsy service and performing research with a focus on digital pathology and tumor pathology.

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## GUEST SPEAKER:

### 11:50-12:30

**Cancer Immunotherapy Challenges and Opportunities: Perspectives from a "Battle-Hardened" Immunologist**

Dr. Laszlo Radvanyi, PhD**;** President and Scientific Director, Ontario Institute for Cancer Research

After completing his PhD in Clinical Biochemistry and Immunology at the University of Toronto, Dr. Radvanyi was granted a Cancer Research Institute Postdoctoral Fellowship at Harvard Medical School in Boston, MA. He then joined Sanofi Pasteur as a Senior Scientist, where he co-led a cancer vaccine antigen discovery and validation team for five years.

Dr. Radvanyi then relocated to Texas where, for ten years, he was a Professor in the Department of Melanoma Medical Oncology at the University of Texas, MD Anderson Cancer Centre. While at MD Anderson, he established a GMP-grade T-cell therapy manufacturing program for metastatic melanoma using expanded tumour-infiltrating lymphocytes (TIL) and performed basic research on TIL biology and effector function for which he received an MD Anderson Division of Cancer Medicine Research Award.

Prior to joining OICR, Dr. Radvanyi was the Senior Vice President and Global Head of the Immuno-Oncology Translational Innovation Platform at EMD Serono (Merck KGaA, Darmstadt, Germany). He also served as a Senior Scientific Advisor for EMD Serono, specializing in immunology and immuno-oncology.

While at EMD Serono, Dr. Radvanyi rebuilt and re-organized EMD Serono's immuno-oncology research platform and rejuvenated their immuno-oncology research pipeline. He recruited key talent and established influential partnerships with academia that focused on biomarker-driven

clinical trials. Notably, Dr. Radvanyi established an alliance between EMD Serono and MD Anderson Cancer Center to perform biomarker-focused clinical trials on key immuno-oncology assets to drive new precision oncology approaches. He also co-led EMD Serono's CAR T-cell program in partnership with Intrexon and Ziopharm at the time.
Dr. Radvanyi also has experience in biotech drug discovery and development (founding CSO for Iovance Therapeutics). Iovance is the first company to develop a commercial process for the manufacturing of TIL and TIL therapy for cancer patients making this a reality after decades of academic research. He was a past Associate Editor for the Journal of Immunotherapy for Cancer (JITC), currently serves on the Keystone Conferences Scientific Advisory Board, and serves on grant review panels for numerous national and international agencies. Dr. Radvanyi also serves as on advisory boards for numerous biotechnology companies in the oncology field.

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## GUEST SPEAKER:

## 3:10-3:55

**The CANcer Detection in Dogs (CANDiD) study: A multi-site, international study to develop a noninvasive "liquid biopsy" test to detect cancer with just a blood draw**

Dr. Andi Flory, DVM, DACVIM (Oncology); Chief Medical Officer, PetDX, University of California (San Diego) Center for Novel Therapeutics

Dr. Andi Flory is a board-certified specialist in Medical Oncology. Dr. Flory graduated from The Ohio State University College of Veterinary Medicine and completed additional training at Florida Veterinary Specialists and Cancer Treatment Center in Tampa, Florida, and Cornell University in Ithaca, NY. Dr. Flory has previously worked as an oncologist in the US and Australia until a little dog named Poppy changed the path of her career and led her to a passion for cancer genomics. In 2019, Dr. Flory co-founded PetDx, in the aim of bringing noninvasive cancer detection to veterinary medicine, so that cancer may be detected earlier.
In 2022, Dr. Flory and her team published the CANDiD study, a clinical validation study of the OncoK9 liquid biopsy test in over 1,000 dogs with and without cancer, which demonstrated an ability to detect 30 types of cancer with a simple blood draw.

Lisa McLennan, SCRA; Director, Clinical Studies & Biobanking, PetDx**,** University of California (San Diego) Center for Novel Therapeutics

Lisa McLennan is a clinical research professional with over 15 years of experience in clinical trial design and oversight. After studying Biology at Montana State University and Towson State University, she began her career in Quality and Risk Management at Maryland General Hospital in Baltimore, MD. After moving back to California, she transferred her abilities to the CRO sector at Quintiles Pacific, where she learned clinical trial design and management. Putting forth her entrepreneurial side, she formed her own S-Corp in 1999 and built a sound reputation as an industry-wide expert in clinical site management for multiple pharmaceutical companies. She took

leave from the clinical research world in 2007 to raise her twin boys. Not one to remain idle, she obtained her Personal Training Certification from the National Institute of Sports Medicine and provided personal training to a select list of clients for a number of years.
In July of 2019, Lisa became part of a small team that founded PetDx with the goal to bring a technology not otherwise present in the veterinary field to light. She and the team designed a clinical trial program that ultimately led to the development of liquid biopsy for dogs.
Outside of work, Lisa enjoys the beach, skiing, crossword puzzles, travelling, and supporting her boys' gymnastics. Her house is blessed by the presence of 3 fur-babies – Beau (a lab/border collie mix), Luna (Super Mutt extraordinaire), and Fergie (our senior orange tabby kitty that rules the roost [named after a whiskey, not Sarah Ferguson or Fergie from Black Eyed Peas]).

## SHORT TALKS FROM INVITED SPEAKERS

**Proteomic profiling of extracellular vesicles from tumour explants as a pipeline for the discovery of biomarkers and targets in canine osteosarcoma**
Dr. Alicia Viloria-Petit, PhD; Associate Professor, Department of Biomedical Sciences, DOGBONe Co-Leader, Ontario Veterinary College, University of Guelph

Dr. Viloria-Petit has a MSc in Immunology (IVIC, Venezuela), a PhD in Cellular and Molecular Biology (University of Toronto, Canada), and more than 25 years of experience in cancer research, including a postdoctoral fellowship at the Lunenfeld-Tanenbaum Institute (Toronto, Canada) where she furthered her interest in signal transduction and metastasis.
Dr. Viloria-Petit joined the Department of Biomedical Sciences at the University of Guelph in 2009. Her research goal is to understand the mechanisms of metastasis and to discover biomarkers and molecular targets to improve diagnosis, prognosis and treatment of advanced mammary and bone cancer in companion animals and humans. Dr. Viloria-Petit's work in canine bone cancer is a key component of the multidisciplinary platform DOGBONe, currently involving 10 researchers from the 4 departments at the OVC.
Besides her work at the University, Dr. Viloria-Petit is committed to promoting gender and ethnicity diversity in STEM, science democratization, and sustainability, to which she dedicates time every week through the Americas-wide program Geeky Latin@s, which she co-founded and co-leads.

**High Intensity Focused Ultrasound (HIFU)**
Dr. Brigitte Brisson, DMV, DVSc, Diplomate ACVS; Professor of Small Animal Surgery, Department of Clinical Studies, Ontario Veterinary College, University of Guelph

Dr. Brigitte Brisson is a Professor of soft tissue surgery in the Department of Clinical Studies at the Ontario Veterinary College (OVC). She graduated from the Faculté de Médecine Vétérinaire at the University of Montreal in 1996. She performed a small animal rotating internship at the

OVC followed by a Surgery residency with concurrent Doctor of Veterinary Science (DVSc) in small animal surgery. She became board certified in small animal surgery (ACVS) in 2001 and has since been on faculty. She is an ACVS Founding Fellow in Minimally Invasive Surgery and a Founding Member of Veterinary Neurosurgical Society.

Her clinical interest is in soft tissue surgery, including oncology and neurosurgery with a specific interest for minimally invasive and interventional vascular and stenting procedures. Dr. Brisson's research has focused on imaging and recurrence of canine intervertebral disc disease and innovative minimally invasive and interventional surgical procedures. She is currently working with a group of pediatric surgeons and interventionalists from the University of Toronto to develop novel treatment options for canine and paediatric cancers. Dr. Brisson has been invited to present the results of her research and other CE around world and has published over 60 articles in refereed journals, authored several book chapters and is the co-editor of Wiley's Current Techniques in Canine and Feline Neurosurgery textbook.

**Modeling osteosarcoma metastasis with PDX-derived cell lines**

Dr. Courtney Schott, BSc, DVM, PhD, DACVP; Assistant Professor, Department of Pathobiology, Ontario Veterinary College, University of Guelph

Dr. Courtney R. Schott is a veterinary anatomic pathologist and cancer researcher. Courtney completed her DVM degree as part of OVC's Class of 2012. She then joined the Department of Pathobiology at OVC and completed her PhD in cancer biology in 2018. Her PhD research focused on predicting outcomes and uncovering mechanisms of chemoresistance in canine osteosarcoma. During her PhD she also completed specialty training in anatomic pathology and became a Diplomate of the American College of Veterinary Pathologists in 2018. After completing her PhD, she commenced postdoctoral studies in the Department of Pediatrics at the University of California San Francisco (UCSF) School of Medicine. Her postdoctoral research focused on pediatric osteosarcoma, investigating mechanisms of metastasis, and developing metastatic models of osteosarcoma. She was awarded a 2020 Rally Foundation for Childhood Cancer Research Postdoctoral and Clinical Research Fellow Grant. In April 2021, Courtney returned to OVC as an Assistant Professor in the Department of Pathobiology. Her research focuses on canine and pediatric osteosarcoma with a particular interest in metastasis and chemoresistance.

## SHORT TALKS FROM SUBMITTED ABSTRACTS

**11:15-11:50 Session One**

### microRNA expression in canine appendicular and metastatic osteosarcoma cell lines and tissues

L. Ludwig, M. Vermey, H. Treleaven, GA. Wood* Department of Pathobiology, Ontario Veterinary College, University of Guelph

Canine osteosarcoma (OSA) is the most common tumour of bone, with most occurring in the appendicular skeleton and euthanasia generally resulting from metastatic disease in the lungs. MicroRNAs (miRNAs) are small, non-coding RNA molecules that bind to mRNA and regulate translation. They are potential diagnostic and prognostic biomarkers in human and canine neoplasms and are found in tissue and extracellular fluids. Previous work in our lab demonstrated that many miRNAs are dysregulated in plasma of OSA patients and are associated with clinical outcomes. To further understand the relationship of miRNAs and OSA we sought to investigate the miRNA profiles of primary appendicular (41 cases) and metastatic pulmonary (12 cases) OSA tissues, along with cell lines (3 cell lines) derived from select tissues. Fifty-nine miRNAs were measured using miRCURY LNA custom PCR panels. Each miRNA was normalized using an average of two endogenous controls and the 2-ΔΔCt method was used to compare normalized Ct values. We found seven miRNAs to be upregulated and four to be downregulated in the primary tumours compared to the pulmonary metastases. Interestingly, miRNA profiles also varied between primary and metastatic cell lines. As well, seventeen miRNAs were upregulated and twelve were downregulated in tissue compared to the matched derived cell line suggesting that both the tumour microenvironment and properties of a rapidly dividing cell line impact the miRNA profile. Analysis is ongoing to investigate the prognostic value of miRNAs in tissue and how these profiles relate to the matched plasma samples previously investigated.

Funding: OVC Pet Trust

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### SHCD adaptor protein modulates EGFT signaling and invasion in breast cancer cells

Hayley Lau[1], Begüm Alural[1], Manali Tilak[1], Ben Staples[1], Laura A. New[1], Kévin Jacquet[2], Nicolas Bisson[2], Jasmin Lalonde[1], and Nina Jones[1]. 1 Department of Molecular and Cellular Biology, University of Guelph, Guelph, ON, Canada. 2 Cancer Research Centre, Quebec Network for Research on Protein Function, Engineering, and Applications (PROTEO) and Centre Hospitalier Universitaire de Québec Research Centre-Université Laval, Québec City, Québec G1R 2J6, Canada

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 signaling complexes to classes of receptor tyrosine kinases implicated in metastatic signaling 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. 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. 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 development/progression. Moreover, we provide a molecular basis for clinical targeting of adaptor-RTK interactions in breast cancer.
Funding: Canadian Research Society

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**Assessing the sexual health needs of patients with prostate cancer accessing a regional cancer centre**

T.M. Kukkonen[1] & M. Preyde*[1] 1. Department of Family Relations and Applied Nutrition, College of Social and Applied Human Sciences, University of Guelph

**Introduction**: Approximately 1 in 7 men will develop prostate cancer in their lifetime, making this the most common cancer diagnosis among males. Although this cancer diagnosis and treatment have been shown to negatively impact sexual functioning, systematic research investigating the sexual health needs of men with prostate cancer remains sparse. As such, the purpose of this research project was to identify the sexual health concerns of patients attending the Grand River Regional Cancer Centre (GRRCC) for the treatment of prostate cancer.
**Methods**: In total, 75 patients (mean age = 73.9 years) attending the GRRCC for treatment of prostate cancer completed an anonymous survey that included open-ended questions and measures of sexual health as well as masculinity.
**Results**: The majority of participants identified as heterosexual (93%) and were within a long-term committed relationship (88%). More than 60% of respondents indicated moderate problems with achieving orgasm. Physical changes in sexual functioning and worries about being able to sexually satisfy their partner were among the top sexual concerns for participants. Finally, participants expressed a desire to obtain more information about the impact of prostate cancer on

their sexual relationships and sexual pleasure, as well as support for their loss of confidence as sexual partners.

**Conclusion**: These findings demonstrate the importance of addressing sexual health for men with prostate cancer and can inform sexual health and relationship programming in psychosocial oncology.

Funding: Research funded by a grant to Dr. Preyde from the Motorcycle Ride for Dads

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**1:35-2:10 Session Two**

**The role of Hippo signalling in OS progression**

Anita Luu[1], Jessica Minott[2], Carey Dougan[3], Lily Chan[2], Khalil Karimi[2], Shelly Peyton[3], Byram Bridle[2], Alicia Viloria-Petit[1]* 1Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph 2Department of Pathobiology, Ontario Veterinary College, University of Guelph 3Department of Chemical Engineering, University of Massachusetts Amherst

Osteosarcoma (OS) is the most common bone tumour in canines and humans. Canines diagnosed with OS undergo the standard of care which includes tumour removal and adjuvant chemotherapy. Despite this treatment, only 20% of dogs will survive 2 years after their initial diagnoses. As such, there is a clinical need to find novel and effective therapeutic candidates in OS. Yes-associated protein 1 (YAP1), one of the downstream effectors of the Hippo signalling pathway, plays a key role in mediating OS progression. Previous work has demonstrated that YAP signalling is important in metastasis, proliferation, and facilitating tumour-stromal interactions within the tumour microenvironment. Based on this, we hypothesized that YAP may be a suitable molecular target in OS. The purpose of this study was to determine the impact of YAP inhibition on the pulmonary metastatic burden and the lung microenvironment, specifically innate immune cell infiltration and mechanical properties.

BalB/C mice were tail vein injected with metastatic murine OS cells or PBS. Mice injected with OS cells were randomly divided to either receive Verteporfin (YAP inhibitor) or DMSO intraperitoneally twice a week. Lung samples were collected on days 4, 8, 16, and 24 for histological, mechanical, and flow cytometry analysis.

Our analysis found that VP reduced the metastatic burden by day 24 and enhanced lung stiffness. Verteporfin increased the number of infiltrated innate immune cells at early time points while decreasing levels at later time points. This preliminary data suggests that YAP inhibition may be beneficial in OS treatment and warrants further investigation.

Funding: Mitacs, OVC Pet Trust

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**2:10-2:50 Session Three**

**A new highly sensitive assay for multiplexed quantification of cancer exosomal proteins in complex biofluids**

Huiyan Li, School of Engineering, University of Guelph

Exosomes play a key role in cancer progression via facilitating intercellular communication. Measuring the protein content of exosomes in biofluids can offer crucial insight into cancer. In order to use these exosomal proteins for cancer management, sensitive, multiplexed, and simple to-use methods are required. Current methods for exosomal protein detection typically require a large sample consumption, and often need an exosome isolation step for complex biofluid samples such as blood plasma. In this work, we have developed a simple and sensitive method of multiplexed detection of cancer protein markers in exosomes, which we call "exosome dot blotting", inspired by conventional dot blotting techniques. After optimization of multiple factors such as antibody concentration, blocking reagent, type of 3D membranes, and use of gold nanoparticles for signal enhancement, cancer-cell-derived exosomes were spiked in pooled normal human plasma for conducting a multiplexed assay in a microarray format. Without the need of isolating exosomes from blood plasma, a limit of detection (LOD) of as low as 4.7 × 104 exosomes/mL or 281 exosomes/sample was achieved, up to four orders of magnitude lower than that of conventional ELISA. This platform offers sensitive, multiplexed, simple, and low-cost exosomal protein detection directly from complex biofluids with only 6 μL of sample consumption, providing a useful tool for multiplexed exosomal protein quantification for a variety of cancer research.

Funding: NSERC, University of Guelph

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**Circulating serum cytokine concentrations of IL-8 and IP-10 have potential prognostic value in canine osteosarcoma.**

N. Hillis[1], A. So[1], S. Patten[2], J.P. Woods[2], G. Wood[3], A.J. Mutsaers*[1,2] 1. Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph 2. Department of Clinical Studies, Ontario Veterinary College, University of Guelph 3. Department of Pathobiology, Ontario Veterinary College, University of Guelph

Osteosarcoma (OSA) is a highly malignant cancer, accounting for 80-90% of primary bone tumours in dogs. Canine OSA patients have a poor prognosis, with approximately 90% of patients dying within 2 years of diagnosis. Standard of care (SOC) treatment consists of limb-amputation with adjuvant chemotherapy. Cytokines mediate communication between the tightly interconnected cells of the bone marrow, including OSA cells when present. Understanding the

role of cytokines in cancer may uncover their potential as prognostic indicators which may help guide treatment. The goal of this study was to investigate serum cytokine profiles of canine OSA patients compared to healthy dogs and in relation to overall survival. Serum samples were collected from 63 appendicular OSA patients at time of diagnosis, 19 of these OSA patients throughout SOC treatment, and 19 healthy dogs. SOC protocol consisted of limb amputation followed by 4 doses of carboplatin every 3 weeks. Cytokine profiles were obtained via Milliplex® MAP Canine Cytokine Magnetic Bead Panel 96-well Plate Assay, which quantified 13 analytes: GM-CSF, IFN-γ, KC-like, IP-10, IL-2, IL-6, IL-7, IL-8, IL-10, IL-15, IL-18, MCP-1 and TNF-α. Mann-Whitney statistical analysis revealed IL-8 and IP-10 were significantly increased in OSA patients compared to healthy dogs, and Kaplan-Meier curve analysis demonstrated high concentrations of IL-8 and IP-10 were significantly associated with shorter survival time. Repeated measures one-way ANOVA showed IP-10 significantly decreased throughout SOC treatment. These results suggest IL-8 and IP-10 may be useful biomarkers in predicting the prognosis of canine OSA patients and are worthy of further validation studies.

Funding: OVC Pet Trust, Art Rouse Scholarship

## INTERACTIVE POSTER SESSIONS
## 10:55-11:15, 1:15-1:35 and 2:50-3:10
## POSTER HALL

**Posters will be available for viewing all day; authors please attend your posters as possible during your designated times. There will be no judging for the poster presentations this year.**

## POSTER ABSTRACTS

**SLN Mapping Agreement in Canine Oral Cancer: A Prospective Study in 42 Dogs**

S. Bae[1], C. Mckenna[2], S. Goldschmidt[3], O. Skinner[4], J. Bertran[5], D. Reynolds[1], and M.L. Oblak[2]
1. Toronto Animal Health Partners; 2. Department of Clinical Studies, Ontario Veterinary College, University of Guelph; 3. Department of Surgical and Radiologic Sciences, University of California – Davis; 4. Department of Veterinary Medicine and Surgery, University of Missouri; 5. Small Animal Clinical Sciences, University of Florida.

Due to the complex anatomy of the lymphocenters of the head and neck and documented interindividual variability in the location of metastatic lymph node(s) (LN), further investigation of Sentinel LN mapping with widely available techniques is a timely topic. This purpose of this prospective study was to investigate the feasibility of indirect CT lymphography (CTL) and intraoperative lymphography with methylene blue (IOL-MB) for SLN mapping in canine oral cancer and to report both agreement between the techniques and accuracy for identifying metastatic LN(s). Forty-two dogs with a primary or incompletely excised oral tumor had routine pre and post contrast CT scans of the head and neck and had CTL, IOL-MB, and tumor/ cervical LN removal. The combination of CTL and IOL-MB techniques yielded an overall detection rate for SLNs of 97.6% with moderate agreement between the modalities (Cohen's kappa = 0.471). Metastatic LNs were identified as the SLN in 6/7 dogs (8/11 LNs). The mean short-long axis ratio was significantly lower when two modalities had the same results than when they did not ($p < 0.05$). A combination of modalities was able to successfully identify a SLN in almost every dog but there were several cases where the SLN varied depending on the modality used. These findings highlight the importance of using a combination of modalities and may indicate that enlarged LN or tumor burden can lead to false negative results and disagreement. This study reinforces that SLN mapping should only be used for subclinical LN metastasis due to the potential effect of metastasis on pressure within the lymphatics.

Funding: OVC Pet Trust

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**Investigating the DEAD-box protein DDX28's regulation of HIF-2α and eIF4E2-directed translation initiation and its role in cancer in hypoxia**

Olivia Bebenek, Doris Tadic and James Uniacke
Department of Molecular and Cellular Biology, University of Guelph.

Hypoxia (low oxygen) is a component of the tumour microenvironment, leading to poorer patient prognosis and resistance to treatments. The Hypoxia Inducible Factors (HIFs) are hypoxia-stabilized transcription factors that regulate the expression of hypoxia-response genes. The lesser-studied α subunit, HIF-2α, is involved in both transcriptional regulation and hypoxic cap-dependent translation, interacting with the hypoxic translation initiation complex that is led by the cap-binding protein eukaryotic initiation factor 4E2 (eIF4E2). It was recently suggested that the DEAD-box RNA helicase protein, DDX28, may act as a tumour-suppressor by inhibiting eIF4E2-directed translation initiation through its interaction with HIF-2α. However, it is currently unknown which of DDX28's motif(s) are necessary for HIF-2α regulation and where in the cell this occurs. We hypothesized that DDX28 interacts with HIF-2α in the nucleus to inhibit it from participating in translation initiation as DDX28 is known to localize to the nucleus and nucleolus, but no function has been investigated. Six EGFP-tagged DDX28 expression plasmids mutated at different motifs and localization signals were created to further define DDX28's regulation of HIF-2α. Additionally, DDX28's normoxic (normal oxygen levels) and hypoxic localizations were investigated through subcellular fractionation. Finally, *DDX28* mRNA and protein levels were surveyed in several cancer cells lines and compared to a non-cancerous cell line in normoxia and hypoxia via qRT-PCR and western blot. By further elucidating DDX28's influence on cancer progression in hypoxia, this research will not only aid in the overall understanding of hypoxic cell processes, but could also lead to the development of novel cancer therapeutics.

Funding: NSERC

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**Pets helping pets (and humans) through innovation and clinical trials**

S. De Paiva, C. McKenna, N. Mashtaler, and M.L. Oblak. Department of Clinical Studies, Ontario Veterinary College, University of Guelph.

The Veterinary Medical Innovation Program is an exciting collaboration between OVC and the Toronto Animal Health Partners and includes the Clinical Translation Platform. The goal of the platform is to support advancement through innovation, facilitating a One Health approach to advancing medical diagnoses and therapies in companion animal health and translating this knowledge to accelerate human medical discoveries. Through consultation with our Scientific Advisory Panel, we take on projects that may involve novel technology, devices or interventions that have a high probability of success and translational impact. Current projects within the

platform include an Artificial Intelligence-based Clinical Management tool and a novel nanomedicine for the treatment of companion animal and human cancers.
Veterinary clinical trials offer a potential solution to the translational gap between preclinical rodent models and human clinical trials. Companion animals, dogs and cats, live in the same environment as their human owners and a result of these similarities, companion animals suffer from diseases with similar etiology. Companion animals provide a high-fidelity naturally occurring disease model that is unprecedented and can help to both evaluate and refine therapy before introduction to humans.
As part of the platform, we are currently actively recruiting canine patients with naturally occurring thyroid tumours for an interventional clinical trial utilizing Porphysomes. This novel nanomedicine has been extensively tested in preclinical animal models but not in naturally occurring cancer patients. Results from this study including optimization of dose and timing and confirmation of limited side effects will inform future studies in companion animals and humans.

Funding: Animal Health Partners Research Chair in Veterinary Medical Innovation, Terry Fox Research Institute

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**Long-term outcome in patients undergoing curative-intent surgery for canine acanthomatous ameloblastomas**

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Canine acanthomatous ameloblastomas (CAA) are benign tumors that can cause significant destruction to underlying bone. Surgical excision with wide margins is the current recommended treatment, however the minimum margin to prevent local recurrence is unknown. The objective of this study was to report local recurrence rates of CAA following curative-intent surgical excision with a minimum follow-up time of 6 months. A secondary outcome was to compare the planned surgical margin to histopathological margins. Fifteen dogs diagnosed with a CAA that underwent surgical excision were included. Median follow-up time was 761 days (242-1909 days). Median age and patient weight was 9.37 years (4.61-12.19 years) and 22.1 kg (5.7-48.7 kg), respectively. Tumors presented primarily in the rostral mandible (60%) with a mean maximum dimension of 2.13 cm (0.2-4 cm). All dogs underwent curative-intent surgery with maximum 2 cm margins. Complications occurred in 7 patients. When evaluating the narrowest bone margin, the mean was 11.7 mm (0-20 mm). Local recurrence was reported in 1 dog, 981 days following surgery. This dog had 1 cm planned surgical margins and clean histologic bone margins of at least 10 mm. Based on these findings, local recurrence following curative-intent surgery for CAA is uncommon and may not correlate with histopathologically clean margins. A definitive cut-off is not possible with this small sample size, but it is likely that 2 cm surgical margins are sufficient to avoid local

recurrence without major post-surgical complications. This study is limited by its retrospective nature, small sample size, and varied follow-up.

Funding: N/A

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**Investigating the tumour microenvironment influence on the hypoxia-induced alternative splicing of eukaryotic ribosomal protein S24**

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A state of hypoxia (low oxygen) is a cellular stressor associated with tumours and is connected to poor prognosis in cancer patients. The stress responses triggered in a hypoxic state provide tumour cells with survival benefits; one such response is hypoxia-induced alternative splicing. The alternative splicing event (ASE) of particular interest occurs in ribosomal protein S24 (RPS24) and is further induced in three-dimensional spheroid models with upwards of a 10-fold induction of the long splice variant. Previous research shows this allows for increased cell survival in hypoxia. This investigation sought to understand the underlying mechanism(s) that further induces the ASE of RPS24 in spheroids with a focus on cell-to-cell contact and apoptosis. The RPS24 long and short splice variants were quantified via qPCR in spheroids treated with ethylene glycol tetra-acetic acid (EGTA) to disrupt the cadherin-junctions between cells. The data suggests the disruption of cell-to-cell contact in spheroids reduces the expression of the long variant, suggesting it's induction in spheroids is due to the increased cell-contact in a tumour microenvironment. Additionally, the RPS24 long and short splice variants were quantified via qPCR in normoxic monolayers treated with staurosporine, an apoptosis inducer. The results suggest the induction of apoptosis induces the expression of the long variant, suggesting it's induction in spheroids is due to the increased occurrence of apoptosis in a tumour microenvironment. Furthering our understanding of the ASE of RPS24 could reveal the underlying mechanisms that increase cell survival during hypoxic stress, leading to the development of novel cancer therapeutics.

Funding Sources: NSERC

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**The EphA2 receptor: a potential therapeutic target in canine and human osteosarcoma**

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Osteosarcoma is a highly metastatic and lethal bone cancer in canines and humans. Although treatment is aggressive, consisting of amputation and chemotherapy, the average duration of

survival is one-year post-treatment for dogs and only 60% of human patients survive longer than 5 years post-treatment. This study aims to better understand the pathophysiology of osteosarcoma with a focus on the role of the EphA2 receptor tyrosine kinase (RTK). EphA2 is one of nine members of the EphA RTK family and in the search for new targeted cancer therapies, EphA receptors are emerging as promising regulators of tumor development, invasiveness, and drug resistance. However, the expression and functional roles of the EphA2 receptor in canine and human osteosarcoma have not been investigated.

Our research has revealed an increased expression of the EphA2 receptor in canine and human osteosarcoma cell lines using Western blotting. To evaluate the EphA2 functions in osteosarcoma cells, we successfully silenced the expression of EphA2 using a specific shRNA. Silencing of EphA2 resulted in reduced proliferation and migration of osteosarcoma cells in culture when compared with non-silenced control cells. Our results also revealed that after EphA2 silencing, osteosarcoma cells showed an increased sensitivity to a common chemotherapeutic drug, Cisplatin, compared to non-silenced cells. In our mouse xenograft model of dog osteosarcoma, silencing of EphA2 also reduced tumor formation in comparison to non-silenced control cells. These data suggest that EphA2 functions as a major driver of the malignant behavior of osteosarcoma in dogs and humans.

Funding: Allard Research Chair Start-up Fund, Companion Animal Health Fund (CAHF) and Saskatchewan Health Research Foundation (SHRF)

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**Determining the effect of intrabody-mediated TERT suppression on cisplatin sensitivity and cellular behavior of human epithelial ovarian cancer cells**

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**Introduction**: Platinum-based therapies are often used to treat epithelial ovarian cancer (EOC). Following initial treatment response, most patients experience resistance and patient relapse. TERT is the catalytic subunit of telomerase. Its main function is telomere maintenance, but it increases proliferation and reduces apoptosis via its telomere-independent functions. Recently, TERT has been associated with chemoresistance; four-fold higher expression was found in resistant compared to chemosensitive EOC cells, and nuclear to mitochondrial translocation was associated with chemoprotection. The following study uses an anti-hTERT intrabody to bind, suppress, and sequester TERT in EOC. We hypothesize this will reduce proliferation, increase apoptosis, and increase cisplatin sensitivity. **Methods**: A constitutive vector expressing an anti-TERT intrabody was constructed. Intrabody expression and nuclear localization were evaluated in normal ovarian surface epithelial (NOSE), and human ovarian cancer (OVCAR-3, and CAOV-3)

cells. A dual-luciferase assay using a TERT promoter-containing firefly luciferase was designed to assess TERT promoter activity, and Southern blotting analysis was used to assess telomere degradation. **Results**: Plasmid construction was confirmed via sequencing. Intrabody expression was confirmed via western blotting and immunofluorescence displaying significant co-localization of nuclear, intrabody, and telomerase signals. CAOV-3 exhibited 72-fold greater hTERT promoter activity than NOSE cells suggesting selective activity and southern blotting analysis showed stable telomere lengths between treated and control cells for all lines. **Conclusions:** Successful intrabody expression and nuclear localization of intrabody was confirmed. The effects of intrabody binding on EOC behavior and chemosensitivity will be studied. This research will help elucidate chemoresistance in EOC and determine the effects of anti-hTERT intrabody expression on EOC cells.

Funding: CIHR, CRS

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**Repurposing simvastatin as a therapeutic strategy in manipulating tumor metabolism in the treatment of high-grade serous epithelial ovarian cancer**

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High-grade serous epithelial ovarian cancer (HGSOC) is a severe clinical problem that necessitates the development of innovative treatments. In a murine ovarian cancer model, we showed metastatic abdominal ascites tumor cells acquire a gain-of-function p53 mutation that drives an upregulation of the mevalonate (MVA) pathway. Signaling through this pathway provided a survival advantage to these cells to fuel their rapid growth and metastasis.
Simvastatin is an inhibitor of HMG-CoA reductase, the rate-limiting enzyme of this pathway, and may strategically regress HGSOC disease. As gain-of-function p53 mutations in fallopian tube epithelial cells is now widely accepted as the origin of HGSOC, we investigated the relationship between p53 status (wildtype, mutant or knockout) and the MVA pathway in oviductal epithelial (OVE) cells, as well as the effect of simvastatin treatment on disease regression. Resazurin, Transwell migration, CyQUANT proliferative and Caspase-Glo 3/7 assays were performed on OVE cells treated with 10 uM simvastatin or DMSO. We developed a novel orthotopic, syngeneic murine model of HGSOC in which p53 mutant OVE cells were injected into the distal oviduct.

Following tumor development, mice were administered PBS or simvastatin daily to assess simvastatin's influence on disease inhibition. Regardless of p53 status, simvastatin significantly decreased metabolic activity over 48 hours. OVE cell invasion was reduced in simvastatin treated p53 mutant cells, relative to p53 wildtype or p53 knockout cells. In all treated OVE cells, simvastatin reduced cell growth and increased apoptotic activity.
In vivo, simvastatin-treated mice had a significant reduction in tumor size and substantial disease regression. Simvastatin inhibits tumorigenesis both in vitro and in vivo. In light of these findings, repurposing simvastatin for the treatment of HGSOC could significantly improve the way we treat this deadly disease.

Funding: CIHR

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**Improving the odds: a novel multi-species approach to cancer research and therapy development**

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Today, 230 Canadians died of cancer. Despite this staggering statistic, we lack confidence in our therapy development pipeline. Our team of basic scientists, veterinary oncologists, and human oncologists offers a bold strategy: a therapy development pipeline that brings all the stakeholders to the table from the beginning and utilizes companion animals (CA) with naturally-occurring cancers as predictive translational models.
While there have been numerous advances in the preclinical setting, with massive advances in genomics, sequencing, human patient-derived xenograft models, and improvements in care, the methodology of drug and therapy discovery has not changed appreciably in almost 5 decades. There is a clear and urgent need to revamp and improve therapy development for our cancer patients – for this we propose a first-in-Canada multi-species translational cancer research program (Improving the Odds) and its translational cancer therapy pipeline (CTP).
The CTP will move novel discoveries from in vitro experimentation to preclinical models to CA cancer patients to human cancer patients. With our unique access to thousands of CA cancer patients, close collaborations with veterinary and human tumor banks, proximity to leading medical institutions, and robust, established collaborations with human clinical scientists, Improving the Odds and the University of Guelph are ideally positioned to lead the nation's collaborative, translational cancer research.

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**In vitro evaluation of optimal concentrations and reconstitution methods of indocyanine green for near-infrared imaging**

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Indocyanine green (ICG) is a near-infrared fluorophore used in various clinical applications. ICG is used for sentinel lymph node mapping (SLN) during surgery for detection of possible metastatic nodes. 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 injection.

Four canine melanoma cell lines (CML1, CML6M, CML10C2, 17CM98) were seeded in 24-well plates with coverslips, to replicate a tissue bed in vitro. The cells were incubated in 400mM ICG that was diluted in saline, dextrose and albumin and fixed in formalin. The cells were labelled with an anti-E Cadherin antibody conjugated to FITC and mounted with DAPI onto slides to be imaged with a confocal microscope. For quantitative analysis, the mean fluorescence intensity was calculated using ImageJ software. This experiment was done in triplicate.

CML1 and 17CM98 incubated with ICG diluted in albumin, dextrose and saline had strong MFI compared to other cell lines and preparations, such as diluting with water ($p < 0.05$). ICG reparations with isotonic or serum solutions result in cell viability post-ICG treatment compared to ICG diluted with water. Analysis histograms depict those 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

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**ICCI Comparative Oncology Program: Utilizing spontaneous companion animal cancers in clinical research studies as models for human cancers**

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Similar to people, cancer is common in companion animals, with ~1 in 3 dogs and 1 in 5 cats developing cancer. The risk of cancer sharply increases over the age of 10, with >50% of pets

developing the disease. Many companion animal cancers share similar characteristics and disrupted pathways to human cancer types. Hence, studies in companion animal cancer patients enable valuable clinical data to be obtained for translational research relevant to human cancer as well as benefiting veterinary patients (e.g. novel techniques and treatment options).
Oncology-related clinical research trials at the Ontario Veterinary College Health Sciences Centre (OVC HSC) are performed with the Institute for Comparative Cancer Investigation (ICCI) which launched in 2007 as the first of its kind in Canada. The ICCI was the first Canadian member in the National Institute of Health-National Cancer Institute (NIH-NCI) Comparative Oncology Trials Consortium (COTC) which conducts international multi-centered studies. Thus far ICCI has collaborated in three trials with COTC investigating novel treatments for osteosarcoma in dogs which is of particular relevance to osteosarcoma in people.
Since 2014, over 1300 client-owned patients have been recruited into 48 oncology-related studies at the OVC. Currently, there are 14 studies recruiting oncology patients: 13 canine and 1 feline (https://icci.uoguelph.ca) investigating several different tumour types and diseases.
Studies in companion animal cancer patients offer the potential to fill the gap that exists between preclinical rodent models and human phase I/II studies. As such, the ICCI comparative oncology program may significantly improve healthcare and lives for both companion animals and people.

Funding Source: OVC Pet Trust and The Smiling Blue Skies Cancer Fund

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**The EphB4 receptor tyrosine kinase promotes the proliferation and migration of canine and human osteosarcoma cells**

Jessica Sharpe, Evelyn Harris, Tim Strozen, Behzad Toosi* Department of Small Animal Clinical Sciences, Western College of Veterinary Medicine, University of Saskatchewan

Osteosarcoma is a highly aggressive bone cancer in canines and humans with a high rate of metastasis to the lungs and a poor prognosis. Current treatments for both species include surgery to remove the primary bone tumor, and chemotherapy or radiation. Advances in treatment options are limited and more effective therapeutic approaches need to be developed.
The Eph receptors are the largest group of receptor tyrosine kinases with nine EphA and five EphB members. The Eph receptors regulate many cellular activities including proliferation, survival, migration, and invasion. Recent evidence suggests that the EphB4 receptor is involved in the regulation of invasion and metastasis of various human cancers. However, the role the EphB4 receptor perform in the fitness of human and canine osteosarcoma has been poorly evaluated. Due to the physiological and cellular similarity between canine and human osteosarcoma, we investigate the role of the EphB4 receptor in promoting osteosarcoma using a comparative approach.

We found upregulated expression of the EphB4 receptor in canine and human osteosarcoma cells as assessed by western blotting. Silencing EphB4 expression using specific shRNAs reduced cell proliferation and migration in both canine and human osteosarcoma and altered colony morphology in human osteosarcoma. Future studies will elucidate the effect of upregulated EphB4 receptor expression on additional cellular functions in vitro including cell invasion and drug sensitivity. The in vivo effect of EphB4 function in osteosarcoma will also be analyzed by testing tumor development and invasiveness in mouse xenograft models.

Funding: Allard Research Chair Start-up Fund, Companion Animal Health Fund (CAHF) and Saskatchewan Health Research Foundation (SHRF)

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**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 Ontario Veterinary College Mona Campbell Animal Cancer Centre, the CATSB is the only veterinary oncology tissue bank in Canada and is registered with two national repository networks. With a current repository of over 1,750 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 29 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

**An investigation of adipocyte-derived mediators of triple-negative breast cancer progression**

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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 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.

In the present study, we conducted liquid chromatography tandem mass spectrometry on conditioned media (CM) of adipocytes cultured in the presence (AE-CM) or absence (A-CM) of overlaid Matrigel. We identified 31 proteins that were exclusively present in the AE-CM and that were not present in Matrigel. Further analysis of cellular localization and literature review revealed six proteins previously shown to be secreted by adipocytes. Of these, we investigated glucosamine-6-phosphate deaminase 1 (GNPDA1) for its capacity to promote partial MET of MDA-MB-231 (a TNBC cell line) in 3D culture, and its secretion by mature adipocytes under different conditions. Our findings suggest that GNPDA1 is one of the mediators of adipocyte-driven MET and should be further investigated in the context of obesity-associated BC.

Funding: Cancer Research Society, NSERC, Memorial University of Newfoundland, and Rouse Memorial Scholarship.

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**A novel mango ginger (Curcuma amada) bioactive, 2,4,6-trihydroxy-3,5-diprenyldihydrochalcone, inhibits mitochondrial metabolism to impart anti-cancer activity in combination with Avocatin B**

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Acute myeloid leukemia (AML) is an aggressive malignancy of the blood and bone marrow in dire new of novel therapeutic options. Nutraceuticals have been identified as promising compounds in the treatment of cancers such as AML; an avocado-derived polyhydroxylated fatty alcohol identified as Avocatin B (Avo B) has been researched by our group for its action in the inhibition of fatty acid oxidation (FAO) in AML. The roots of the mango ginger plant, Cucuma amada, have been used in traditional medical practices and are closely related to turmeric. A bioactive molecule identified as 2,4,6-trihydroxy-3,5-diprenyldihydrochalcone (M1) isolated from C. amada was investigated in this study for its anti-leukemic effects both independently and in combination with Avo B.

M1 (0.16 - 0.63 μM) and Avo B (2.0 μM) synergistically reduced leukemia cell growth. Combination index (CI) values, as determined using Calcusyn software, were 0.6, 0.65, and 0.8, which indicate statistical synergy (i.e., CI<1). Antagonistic combinations (CI>1) were noted with doxorubicin and cytarabine. In cells resistant to FAO, the combination was ineffective at reducing leukemia cell viability but M1 remained active. Mechanistically, M1 was further shown to inhibit complex I of the mitochondrial complex chain, suggesting that both molecules target altered leukemia cell metabolism. Taken together, M1 and Avo B is a novel combination with activity in AML worth of further exploration.

Funding: University Health Network, Ministry of Agriculture, Food & Rural Affairs, Ontario Institute of Cancer Research