ONTARIO VETERINARY COLLEGE | INSTITUTE FOR COMPARATIVE CANCER INVESTIGATION

16th Annual ICCI Cancer Research Symposium

Thursday May 23, 2024

OVC ECLA 1720 9:00-5:00

Introductory Remarks

Welcome to the 16th annual ICCI Cancer Symposium! We are so excited to welcome you all in person again this year. 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. Chand Khanna is the 2024 Arthur Willis Distinguished speaker and will be giving the keynote address at 3:00 p.m.

In the past 16 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 getting you all here today. We also thank Alicia Viloria-Petit, Charly McKenna, Bridget Bane and our volunteer team, the OVC administrative assistants and communications team for their extensive help with information dissemination, and our University of Guelph Information Technology team for help with the live stream and recordings. The research projects presented here and the trainees performing these studies were collectively supported by grants, scholarships and contracts from: Canadian Cancer Society, Canadian Institutes of Health Research, Cancer Research Society, Health Canada, Leukemia & Lymphoma Society of Canada, Mitacs, Natural Sciences and Engineering Research Council of Canada, Ontario Centres of Excellence, Ontario Institute of Cancer Research, Ontario Veterinary College, OVC Pet Trust, Pathobiology Grad Growth, SickKids Foundation, Smiling Blue Skies Cancer Fund, University of Guelph, Vanier Canada Graduate Scholarship, and Zoetis.

ICCI 16th Annual Cancer Research Symposium, Thursday May 23rd, 2024

Morning Session

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

9:05-9:35 Guest Speaker. Moderator: Geoff Wood
Studies of metabolism in canine osteosarcoma
Dr. Tony Mutsaers; Department of Biomedical Sciences, University of Guelph

9:35-10:15 Short talks from abstracts (*Biomarkers*). Moderator: Alicia Viloria-Petit

1. *3D Compartmentalized linker array for low-cost and highly sensitive multiplex detection of cancer-related proteins*
   Roshan Tosh Aggarwal; School of Engineering, University of Guelph

2. *Highly sensitive multiplexed detection of cancer-related biomarkers using gold-nanoparticle-embedded membrane*
   Rebecca Goodrum; School of Engineering, University of Guelph

3. *Multiple microRNA models in serum and tissue for screening and diagnosis of splenic masses in dogs*
   Latasha Ludwig; Department of Pathobiology, University of Guelph

10:15-10:35 Biobreak – Please take this opportunity to visit our posters in the cafeteria while you enjoy a refreshment, or see more on our website at https://sites.uoguelph.ca/icci/icci-annual-symposia/2024-icci-cancer-research-symposium/2024-icci-symposium-posters/

10:35-11:15 Short talks from abstracts (*Gene expression*). Moderator: Geoff Wood

4. *Longitudinal study of transcriptomic changes occurring over initial six weeks of CHOP treatment in canine lymphoma (Remote presentation)*
   Miles Mee; Department of Biomedical Sciences, University of Guelph

5. *Expression of c-Met receptor for tumour-specific near-infrared imaging in human and canine non-small cell lung cancer*
   Ann Steffi Ram; Department of Biomedical Sciences, University of Guelph

6. *Targeting the Notch signaling pathway to improve osteosarcoma chemotherapy response by reducing stem-like characteristics*
   Aidan Russel; Department of Biomedical Sciences, University of Guelph

11:15-11:45 Guest Speaker. Moderator: Geoff Wood

Bench to Bedside Overview

Dr. Michelle Oblak/Charly McKenna; Department of Biomedical Sciences, University of Guelph

Due to unforeseen circumstances, our regional keynote, Dr. Abdul Razak, has had to cancel his talk. Thanks to Dr. Oblak and Charly McKenna for stepping into this timeslot.

11:45-1:30 ***Lunch Break and Poster Viewing – food will be served in 1720 and can be enjoyed at your seat or outside in one of the seating areas.***
Please take some time to visit the posters in the cafeteria and speak with the presenters at this time. Judging of the posters will be taking place, so if judges are present at the poster, please allow presenters to finish with the judges before asking questions.

Afternoon Session

1:30-2:00 Guest Speaker. Moderator: Michelle Oblak

Hematopoietic Neoplasia: Current State and What Lies Ahead

Dr. Dorothee Bienzle; Department of Pathobiology, University of Guelph

2:00-2:40 Short talks from abstracts (*Immune, Modeling*). Moderator: Geoff Wood

7. *Developing a type I conventional dendritic cell vaccine for glioblastoma*
   Shayla Verburg; Department of Pathobiology, University of Guelph

8. *Tumour associated neutrophils, circulating neutrophil counts, and circulating lymphocyte counts predict poor outcome in canine appendicular osteosarcoma*.
   Rachael Speare; Department of Pathobiology, University of Guelph

9. *Examining in vivo-in vitro correlations in osteosarcoma metastasis models*
   Emma Vanderboon; Department of Pathobiology, University of Guelph

2:40-3:00 Biobreak – Please take this final opportunity to visit our posters in the cafeteria. Online posters will be available until the end of the day today. Select posters will remain on our website with presenter consent.

3:00-4:00 **Keynote Speaker.** Moderator: Paul Woods

A Comparative and Iterative Approach to Osteosarcoma Metastasis: Biology and Therapy

Dr. Chand Khanna; Founder and Board Chair of Ethos Discovery

4:00-5:00 Closing Remarks, Awards Presentations and Light Reception

KEYNOTE PRESENTATION

3:00 – 4:00 p.m.

Dr. Chand Khanna, DVM, PhD, DACVIM

Founder and Board Chair of Ethos Discovery

A Comparative and Iterative Approach to Osteosarcoma Metastasis: Biology and Therapy

---

Dr. Chand Khanna is the founder and board chair of Ethos Discovery, a non-profit incubator of scientific innovation with a deep interest in solving complex medical problems and improving patient outcomes.

Dr. Chand Khanna is a graduate of the Western College of Veterinary Medicine, in Saskatoon. He then received specialty training in the fields of veterinary internal medicine and oncology, first at the Ontario Veterinary College, University of Guelph, and then at the University of Minnesota. Dr. Khanna is a Diplomate of the American College of Veterinary Internal Medicine (Oncology). Following this clinical specialization, Dr. Khanna received a Ph.D. in Pathobiology from the University of Minnesota and then completed a post-doctoral fellowship with Dr. Lee Helman in the Pediatric Oncology Branch of the National Cancer Institute in Bethesda, Maryland. He was recently awarded honorary membership as a Diplomate of the American College of Veterinary Pathology.

Following his post-doctoral fellowship, Dr. Khanna continued his work at the National Cancer Institute as the Head of the Pediatric Oncology Branch's Tumor and Metastasis Biology Section and Founding Director of the Center for Cancer Research, Comparative Oncology Program. In 2011, Dr. Khanna was granted full tenure and promoted to the position of Senior Investigator at the National Cancer Institute. His research interests and responsibilities focused on the problem of cancer metastasis and the development of new options to treat patients with metastasis. He has over 100 publications in the area of cancer biology and therapy. He is the editor of a recently published textbook entitled, "Therapeutic Strategies in Veterinary Oncology

Dr. Khanna has held leadership roles in both veterinary and human oncology, including, president of the American College of Veterinary Internal Medicine, chair of the Children's Oncology Group Bone Biology Subcommittee, Director of the SARC (Sarcoma Alliance for Research through Collaboration) Developmental Therapeutics Committee. Dr. Khanna is a founding member of the

Canine Comparative Oncology and Genomics Consortium. Dr. Khanna is a two-time recipient of the NCI Distinguished Mentor Award and was the 2010 recipient of the NCI Award for Outstanding Research. Dr. Khanna currently serves on the boards of biotechnology companies involved in veterinary oncology and metastasis drug development and is Chair of the Strategic Advisory Board of the Osteosarcoma Institute.

Despite many roles throughout his professional career, Dr. Khanna self-identifies as a clinician first. He was an active clinician within his referral oncology practices, The Oncology Service, based in the greater Washington, D.C. area, until 2021. He is also the founder of Animal Clinical Investigation, a contract research company involved in complex medical problems seen in pets.

Past ICCI Symposium Arthur Willis Distinguished Speakers

2023	Amy LeBlanc	2017	William Eward	2013	David Argyle
2022	Elizabeth Murchison	2016	Jaime Modiano	2012	Timothy Fan
2021	Lisa Forrest	2015	Nicola Mason	2011	Cheryl London
2019	David M. Vail	2014	Deborah Knapp	2010	Matthew Breen
2018	Daniel Gustafson				

GUEST SPEAKER:

9:05 - 9:35

Studies of metabolism in canine osteosarcoma
Anthony J. Mutsaers, DVM, PhD, Diplomate ACVIM (Oncology); Departments of Biomedical Sciences and Clinical Studies, University of Guelph

Dr. Mutsaers graduated with a DVM from the Ontario Veterinary College. Following a residency in comparative oncology at Purdue University he is a diplomate of the American College of Veterinary Internal Medicine in Oncology. He received his PhD in Medical Biophysics from the University of Toronto, then completed a post-doctoral fellowship in the Stem Cell Regulation Unit, St. Vincent's Institute of Medical Research, Melbourne, Australia, studying osteosarcoma. Dr. Mutsaers then joined the University of Guelph and is currently an Associate Professor in the Departments of Clinical Studies and Biomedical Sciences. His laboratory uses comparative oncology to translate discoveries from preclinical investigation to clinical application through naturally occurring cancers in dogs, with a focus on sarcoma, melanoma, and bladder cancers.

GUEST SPEAKER:

11:15 – 11:45 – Cancelled

~~**Sarcoma Clinical Trials in Ontario: Advances, Challenges and Opportunities**~~
~~Albiruni A Razak, MB, MRCPI, CCT (Medical Oncology); Princess Margaret Cancer Centre, Mount Sinai Hospital and University of Toronto~~

Bench to Bedside Overview
Michelle Oblak, DVM, DVSc, Diplomate ACVS, ACVS Fellow of Surgical Oncology, and Charly McKenna, RLAT, BSc, MSc, PhD Student; Department of Clinical Studies, University of Guelph

---

GUEST SPEAKER:

1:30 – 2:00

Hematopoietic Neoplasia: Current State and What Lies Ahead
Dorothee Bienzle, DVM, MSc, PhD, Diplomate ACVP; Department of Pathobiology, University of Guelph
Dorothee Bienzle is a Clinical Pathologist with particular interest in hematolymphoid pathology.

She has a DVM from the University of Guelph and a PhD in immunology from McMaster University, and has been on the faculty of the Universities of Georgia and Guelph. Dorothee has guided the training of over 20 graduate and 30 undergraduate students, respectively, and has researched and published extensively in the area of veterinary hematolymphoid pathology. Her contributions have been recognized with multiple awards.

Lymphoma and leukemia are heterogeneous cancers in dogs and cats, as they are in humans. Tools available for diagnosis and characterization of different subtypes have expanded in the last decade from cytopathology, histopathology, clonality and immunophenotyping to include sequence-based genetic analysis. Microscopic interpretations are largely standardized following algorithms validated for animal neoplasms, and there has also been progress in immunophenotyping through use of comprehensive panels and standardization of analysis and interpretation. While genetic analysis has tremendous potential due to species independency in method, meaningful interpretation is complex and evolving. Diagnostic approaches are ideally integrated within a biologically meaningful and economically feasible framework in a stepwise fashion, with incorporation of epidemiological and clinical patient characteristics. This presentation will provide a brief summary of current and future diagnostic methods in the context of the biology of hematolymphoid neoplasms in companion animals.

SHORT TALKS FROM SUBMITTED ABSTRACTS

9:35 - 10:15 Session One

3D Compartmentalized Linker Array for Low-Cost and Highly Sensitive Multiplex Detection of Cancer-Related Proteins

Roshan Tosh Aggarwal, Rebecca Goodrum, and Huiyan Li*. School of Engineering, University of Guelph

Cancer is a complex disease at the molecular level. Detecting and understanding biomolecular makeup is vital for improved diagnosis and intervention. Microarray bioassays allow simultaneous analysis of multiple biomolecules with reduced costs and materials. However, its use has been limited due to fabrication complexity and cost. Furthermore, the mixing of different bioreagents in a multiplexed assay leads to cross-reactions, producing false positive signals which impair assay reproducibility and scalability.

A Nitrocellulose Compartmentalized Linker Array (nCLA) that consists of pre-prepared storable microarrays on nitrocellulose membranes in microliter compartments is created with a novel selective blocking technique. nCLA can be used for binding and patterning bioreagents into

microarrays by simply pipetting and incubating microliters of bioreagent solutions in the compartments, instead of printing nanoliters of bioreagent with a complex microarray printer. The nCLA successfully measured three cancer-related proteins in a multiplexed sandwich immunoassay while only using 2 μL of reagent per compartment for measuring three markers, 150 times less than a standard ELISA for each marker. The nCLA's limits of detection for EGFR, TNF-α, and GM-CSF are 1.9 pg/mL, 1.4pg/mL, and 1.3 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. The nCLA was also used for detecting these proteins in human plasma.

Using low-cost materials and equipment, the nCLA provides a simple platform for making multiplex assays widely available and enables the broader adoption of microarrays in cancer research. Future work will seek to augment the nCLA assay signals using gold nanoparticles and investigate its utility in extracellular vesicle detection.

Funding: Natural Sciences and Engineering Research Council of Canada (NSERC), University of Guelph

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

Highly sensitive multiplexed detection of cancer-related biomarkers using Gold-nanoparticle-embedded membrane

Rebecca Goodrum, Roshan Tosh Aggarwal, Huiyan Li*. School of Engineering, University of Guelph.

Quantifying proteins from biofluids can offer critical insight into health for the diagnosis and prognosis of diseases such as cancer. During early-stage cancer, many of the disease-related protein biomarkers are present in low concentrations and require highly sensitive biosensing methods to detect. Tumors are very small in these early stages and with current methods they can grow for 10 years before being detected. Cancer, along with many other diseases, is complex and involves multiple protein biomarkers, thus, multiplexed detection of biomarkers is important for accurate diagnosis. Current technologies lack sensitivity, multiplexing capabilities, or require complicated micro/nano-fabrication processes which limit their use. Gold nanoparticles (AuNPs) interact with fluorescent molecules to cause metal enhanced fluorescence (MEF) and 3D nitrocellulose membranes have increased binding capacity, both of which work to enhance sensitivity. Here, a compartmentalized gold-nanoparticle-embedded membrane (GEM) platform, which combines these properties is presented. With optimized size, shape, and concentration of AuNPs to achieve optimal MEF, its application for multiplexed sandwich immunoassays for the quantification of three cancer-related proteins in blood plasma has been demonstrated. With up to 20-fold less sample consumption, sensitivity was improved by 9-folds, 287-folds and 949-folds for EGFR, GM-CSF, and TNF-α respectively compared to conventional ELISA. In spiked human

plasma, low pg/mL sensitivity was also achieved. This user-friendly platform offers sensitive and multiplexed detection capabilities with low sample consumption, providing a useful tool suitable for a variety of applications, including early disease diagnostics.
Funding: Natural Sciences and Engineering Research Council of Canada (NSERC), University of Guelph

---

Multiple microRNA models in serum and tissue for screening and diagnosis of splenic masses in dogs

Latasha Ludwig[1], Heather Treleaven[1], Arlene Khachadoorian[1], Brigitte Degasperi[2], Ingrid Walter[3], Deirdre Stuart[4], Roger Moorehead[5], Robert A. Foster[1], R. Darren Wood[1], Ayesha Ali[6], Geoffrey A. Wood[1]. 1. Department of Pathobiology, University of Guelph; 2. Department for Small Animals and Horses, University of Veterinary Medicine, Vienna; 3. VetCore Facility for Research, University of Veterinary Medicine, Vienna; 4. Veterinary Biobank, Department of Clinical Studies, University of Guelph; 5. Department of Biomedical Sciences, University of Guelph; 6. Department of Mathematics and Statistics, University of Guelph.

Splenic masses are common in dogs and vary dramatically in their clinical behaviour. Hemangiosarcoma (HSA), the most common malignancy of the spleen, is a very aggressive tumour with a poor prognosis. Clinically, it is difficult to differentiate between benign and malignant splenic masses. Although histopathology is the gold standard for diagnosis, even with this method it can be challenging to provide an accurate diagnosis due to sampling limitations. MicroRNAs (miRNAs) are small RNA molecules present in all cells and blood. They are differentially expressed in numerous cancers in dogs and humans. We hypothesize that miRNAs in tissue and serum can differentiate between HSA and other splenic masses. Fifty-nine miRNAs were investigated by RT-qPCR (QIAGEN miRCURY arrays) in serum and/or tissue from dogs with HSAs, lymphomas, non-angiomatous, non-lymphomatous sarcomas, histiocytic sarcomas, benign splenic masses (myelolipomas, nodular hyperplasia, hematomas), and healthy dogs. Numerous miRNAs were differentially expressed between HSA serum and tissue compared to other splenic masses and dogs with normal spleens. In serum, our six-miRNA model (miR-135a-5p, miR-16-5p, miR-10a, miR-450b, miR-152-3p, and miR-126-5p) accurately classified 100% of HSA cases from healthy dogs and those with a benign splenic mass. The overall accuracy of the model was 88.14%. In tissue, our three-miRNA model (miR-126-5p, miR-502-3p, and miR-452-5p) accurately classified 96.15% of the HSAs and benign splenic masses evaluated. This study demonstrates the utility of multiple miRNA models in serum and tissue for screening and diagnosis of HSA in dogs. Future work includes evaluation of prospective and pre-diagnosis serum samples.
Funding: OVC Pet Trust, OVC Fellowship and NSERC Vanier CGS

10:35 - 11:15 Session Two

Longitudinal study of transcriptomic changes occurring over initial six weeks of CHOP treatment in canine lymphoma

M. W. Mee[1], S. Faulkner[1], G. A. Wood[2], J. P. Woods[3], D. Bienzle[2], B. L. Coomber[1]*. 1. Department of Biomedical Sciences; 2. Department of Pathobiology; 3. Department of Clinical Studies and Mona Campbell Center for Animal Cancer, Ontario Veterinary College, University of Guelph

Canine lymphoma (CL) is the most common hematological cancer in dogs. The standard treatment for CL is the CHOP chemotherapy protocol consisting of cyclophosphamide, doxorubicin (hydroxydaunorubicin), vincristine (Oncovin), and prednisone. Most CL patients treated with CHOP initially achieve remission but the majority eventually relapse with a multi-drug resistant phenotype. This study assessed changes in gene expression occurring in CL cells over the initial six weeks of CHOP treatment using RNA-Seq data from 15 matched diffuse B-cell lymphoma patient samples taken prior to treatment and again six weeks into treatment. We identified 295 genes significantly differentially expressed; 123 were up-regulated and 172 were down-regulated (FDR [false discovery rate] adjusted p-value < 0.05). The up-regulated genes included the multi-drug resistance associated drug efflux transporter ABCB1. The down-regulated genes were enriched for those involved in cell cycle and DNA repair, and for those targeted by E2F transcription factors. E2F3 was the only E2F transcription factor significantly downregulated (FDR adjusted p-value < 0.05). After dividing the patients into two groups, based on median E2F3 expression at the 6-week time point, t-SNE dimensionality reduction of the gene expression profiles showed the groups to be interspersed at the pre-treatment time point and clustered separately at the 6-week timepoint. These results suggest a selection for CL cells with down-regulated expression of cell cycle and DNA repair genes in response to CHOP therapy, and that this down-regulation occurs through the regulation of E2F transcription factors like E2F3.

Funding: Ontario Centres of Excellence

Expression of c-Met receptor for tumor-specific near-infrared imaging in human and canine non-small cell lung cancer

A. S. Ram[1]*, J. Petrik[1], G. A. Wood[2], S. Workenhe[3], and M. L. Oblak[3]. 1. Department of Biomedical Sciences; 2. Department of Pathobiology; 3. Department of Clinical Studies, Ontario Veterinary College, University of Guelph.

Non-small cell lung cancer (NSCLC) is the most prevalent subtype of lung cancer and surgical resection is the initial standard of care. To prolong patient survival and maximize preservation of healthy tissue, intraoperative guidance is necessary to delineate malignant and healthy tissue. Recently, near-infrared (NIR) imaging has been utilized to improve intraoperative tumor bed imaging with high efficacy, however there is a lack of specific tumor targeted NIR imaging agents

for NSCLC. We aim to utilize overexpressed receptors in NSCLC to develop an NIR imaging probe that can provide reliable surgical resection guidance.
Three human NSCLC cell lines (A549, NCI-H1975, and NCI-H358), three canine lung adenocarcinoma cell lines (CLAC, HDC, and LuBi), and a normal human small airway epithelial cell line were used. Western blots and immunofluorescence microscopy was performed. All experiments were done in triplicate. Immunohistochemistry of tumor and paired normal NSCLC tissues was also performed on a human and canine tissue microarray.
Based on protein densitometric analysis and microscopy, c-Met was significantly overexpressed in human NSCLC cells compared to normal cells ($p < 0.005$). In canine lung cancer cells, c-Met was expressed at similar levels to A549 ($p = 0.1028$). Relative to normal tissue, c-Met has a higher expression in lung tumor tissue ($p < 0.005$) and this observation was conserved between the two species. These results depict that c-Met may be an optimal imaging target for NSCLC due to the overexpression and distribution of c-Met receptors in tumor tissue compared to normal tissue.
Funding: OVC Pet Trust

Targeting the Notch signaling pathway to improve osteosarcoma chemotherapy response by reducing stem-like characteristics

Aidan Russell[1], Anthony Mutsaers[1,2]*. 1. Department of Biomedical Sciences; 2. Department of Clinical Studies, Ontario Veterinary College, University of Guelph.

Osteosarcoma is the most common primary bone cancer in humans with a localized disease survival rate of approximately 75%, which drops significantly after recurrence and/or metastasis. No further improvements in outcome have been made since the introduction of neoadjuvant chemotherapy and surgery. Cancer stem cell (CSC) theory posits that a small portion of cancer cells can regenerate the bulk of the tumor, possess chemo-resistance mechanisms, and enhanced migration/metastatic capabilities. One potential target which has been shown to help maintain CSCs is the Notch signaling pathway. MK-0752 is a small molecule gamma secretase inhibitor that blocks the cleavage of the Notch intracellular domain and the resulting downstream signaling. To investigate whether inhibition of Notch signaling can improve chemotherapy response, commercially available human osteosarcoma cell lines were grown in conventional monolayer and adhesion-free serum starved conditions as spheroids, which enrich for CSCs. Gene expression analyses comparing monolayer and spheroid cell cultures showed increased Notch pathway genes Notch1 receptor, Hes1 and Hey, as well as increased stem cell markers ABCG1 and Sox2 within spheroids. In single-agent cell viability experiments, a high IC50 was found for MK-0752 treatment of both monolayer cells and spheroid cells with spheroids having a lower IC50, while no difference in IC50 was observed with doxorubicin. In MK-0752 + doxorubicin combination treatment experiments, synergism was observed at all doses. MK-0752 in combination with doxorubicin is one possible combination that has improved chemotherapeutic

response. Targeting the Notch signaling pathway is worthy of further investigation for improving chemotherapy response in osteosarcoma treatment.
Funding: OVC Scholarship

2:00 - 2:40 Session Three

Developing a type I conventional dendritic cell vaccine for glioblastoma

S. Verburg[1], J. Inkol[1], M. Westerveld[1], S. Walsh[1], J. Geddes-McAlister[2], J. McAlister[2], D. Brewster[3], S. Workenhe[1]*. 1. Department of Pathobiology, Ontario Veterinary College; 2. Department of Molecular and Cellular Biology; 3. Advanced Analysis Center, University of Guelph.

Glioblastoma (GBM) is considered one of the most lethal human malignancies. Due to the altered immunology, failure of current therapies and dismal survival, new treatments are needed. Dendritic cell (DC) vaccines show promise in generating anti-tumor immune responses against GBM; however, the specific DC subset and optimal maturation signals remain unexplored. Our recent observations have shed light on the ability of cytotoxic treatments to active type I conventional dendritic cells (cDC1s), suggesting a possible avenue for enhancing anti-cancer T-cell responses. However, it remains unknown what type(s) of programmed cell death are efficient at activating cDC1s to prime anti-cancer T-cells. We hypothesize that, unlike apoptosis, both necroptosis and pyroptosis promote the release of distinct secretomes that differentially regulate cDC1-mediated antigen presentation to T-cells.
In this study, I utilized our engineered murine GBM cells that inducibly undergo three forms of cell death. Cell-free supernatants were harvested from dying and control cells to quantify the secretomes by mass spectrometry. To define how cell death shapes cDC1 functionality, I used an ex-vivo DC and GBM cell co-culture system. GBM cells undergoing necroptosis, pyroptosis and apoptosis were added into a cDC1 co-culture, and flow cytometric analysis revealed differences in their activation and maturation states. Future studies will aim to uncover the role of cDC1's primed by cell death secretomes on T-cell activation by generating a cDC1: T-cell co-culture in the presence of dying GBM cells. The impact of these studies is to identify GBM secretome capable of stimulating efficient cDC1-mediated anti-tumor T-cell responses.
Funding: OVC Pet Trust; OICR; Cancer Research Society; SickKids Foundation; CIHR

Tumour associated neutrophils, circulating neutrophil counts, and circulating lymphocyte counts predict poor outcome in canine appendicular osteosarcoma.

R. Speare[1], V. Huntley[2], J.R. Fischbach[1], C.R. Schott[1], R.D. Wood[1], G.A. Wood[1], A. Viloria-Petit[2]*. 1. Department of Pathobiology; 2. Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph.

Osteosarcoma is the most common bone tumour of humans and pet dogs. Improvements in prognostication would be beneficial to both species. High circulating neutrophil count correlates with poorer outcome in humans and dogs. High neutrophil to lymphocyte ratio (NLR) correlates with poorer outcome in humans. Tumour infiltrating lymphocytes have been evaluated in both species; tumour associated neutrophils (TANs) have yet to be investigated. This study aimed to uncover correlations between outcome and circulating lymphocytes and neutrophils, as well as TANs in canine appendicular osteosarcoma patients receiving standard of care treatment. Circulating lymphocyte, segmented neutrophil (SN), and band neutrophil (BN) counts were recorded for 76 pre- and 86 post-amputation patients. TANs were quantified using myeloperoxidase immunohistochemistry in 34 cases. Kaplan-Meier curves for disease-free interval (DFI) and survival time (ST) were compared by log rank test. High lymphocyte count correlated with shorter DFI pre- and post-amputation ($p<0.05$). High SN count correlated with shorter DFI and ST pre-amputation ($p<0.05$). An increase in total neutrophil count (SN+BN) over 2.23 from pre- to post-amputation correlated with longer DFI ($p<0.05$). BN count and NLR did not correlate with DFI or ST. TAN counts ranged from 0/mm^2 - 4.86/mm^2, and TAN count above 0.58/mm^2 correlated with shorter DFI ($p<0.05$). The relationships identified support and expand upon previously reported findings in humans and dogs. TAN values in canine osteosarcoma are described for the first time. Future work in dogs can provide insights into mechanisms underlying the role of inflammation in osteosarcoma tumour progression and metastasis across species.
Funding: OVC Pet Trust

Examining in vivo-in vitro correlations in osteosarcoma metastasis models.

Emma N. Vanderboon and Courtney R. Schott*. Department of Pathobiology, Ontario Veterinary College, University of Guelph.

Osteosarcoma is the most common primary malignant bone tumour and is notorious for its high rate of metastasis. *in vitro* assays that mimic the steps of metastasis represent an important model to enhance our understanding of this process and to predict neoplastic cell behaviour; however, *in vivo* models remain the gold standard. This study aims to develop an *in vitro* assay pipeline to investigate mechanisms of metastasis and to characterize a panel of human osteosarcoma cell lines previously characterized *in vivo*, allowing us to examine *in vitro- in vivo* correlations. Cell line proliferation, migration, and invasion, as well as capacity for anchorage independent survival and colony formation will be evaluated *in vitro*. The osteosarcoma cell lines SJSA and MG63 exhibit contrasting metastatic behaviour in both orthotopic and intravenous murine models. SJSA displays high metastatic burden, whereas MG63 shows limited metastasis in these murine models, and their *in vitro* behaviour is compared here. *in vitro*, SJSA displays a superior ability to migrate within scratch and transwell assays, aligning with its *in vivo* metastatic phenotype. However, MG63 exhibits a faster doubling-time and more efficient colony formation

representing a lack of direct correlation between *in vivo* vs. *in vitro* behaviour. Further characterization of these cell lines and an entire human osteosarcoma cell line panel will reveal which *in vitro* assays correlate best with *in vivo* behaviour in murine models of metastasis, facilitating more biologically relevant extrapolations to be made from future mechanistic studies utilizing this *in vitro* pipeline.

Funding: OVC Pet Trust & Zoetis

POSTER ABSTRACTS

In person posters will be available for viewing all day in the OVC cafeteria; questions can be answered by the presenters during the live poster session. Remote presenters will have their posters available on the ICCI website, and can be contacted by email where indicated. Following the close of the symposium, presenters may have their posters available on the ICCI website for future viewing at https://icci.uoguelph.ca/icci-annual-symposia/2024-icci-cancer-research-symposium/2024-icci-symposium-posters/.

---

1. Comparison of Normalization Methods for RT-qPCR microRNA Expression in Cancer Datasets

H. Treleaven[1], L. Ludwig[1], A. Viloria-Petit[2], R.D. Wood[1], R.A. Ali[3], G.A. Wood[1]*. 1. Department of Pathobiology, Ontario Veterinary College, University of Guelph 2. Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph 3. Department of Mathematics and Statistics, University of Guelph

Real-time quantitative PCR (RT-qPCR) is a widely used method for quantifying microRNA (miRNA) expression, but its accuracy depends on appropriate normalization. RT-qPCR is susceptible to technical variation from several sources, including sample collection and storage, miRNA quantity/quality, and extraction and amplification efficiencies. Normalization corrects for these factors, theoretically leaving behind only biological variation. With growing interest in miRNAs as biomarkers for the diagnosis and prognosis of various cancers, analyses must be accurate and consistent. Primarily, the comparative Ct method is used, which compares the expression of miRNAs to a reference value. Unfortunately, there is a lack of consistency on what to use as a reference. A standard recommendation is the average of multiple, stable miRNAs referred to as endogenous controls (ECs). Currently, there are no known universally stable miRNAs. Therefore, ECs should be selected on a per-disease and per-sample type basis. Multiple algorithms exist to

identify stable ECs, but few studies indicate what, if any, steps were taken to implement them correctly. Also, many studies have used non-ideal reference values such as small nuclear RNAs or a single miRNA. This lack of agreement on protocol often leads to difficulty comparing studies and may produce incorrect results. This work addresses these inconsistencies by making recommendations for the normalization of RT-qPCR miRNA expression. We compared 2 widely used methods for EC selection, NormFinder and GeNorm, and to show how normalization influences results we also used an unstable miRNA. Only miRNAs with expression in all samples were considered as potential ECs. GeNorm is sensitive to correlated genes. Therefore, for any pair or trio of correlated miRNAs 1 was kept. NormFinder performs optimally with 5-10 candidate genes; therefore, we tested the 10 miRNAs with the lowest coefficients of variation (CV). The top 3 stable miRNAs were selected from each algorithm to serve as ECs, and we used their average expression to normalize each sample. For the single miRNA, samples were scaled based on its value. We applied these methods to 3 independent datasets: First, plasma samples from a study on canine osteosarcoma (OSA), including pre-amputation (n=45), post-amputation (n=27), and healthy controls (n=21). Second, tissue samples from a study on canine OSA, including primary tumour (n=42) and lung metastases (n=12). Third, serum samples from a study on canine lymphoma included B-cell (n=24), T-cell (n=16), and healthy controls (n=14). For datasets 1 and 3, NormFinder provided a better reduction in gene-specific CV and a more favourable cumulative distribution of the CV. For dataset 2, there was less initial variability, but NormFinder still had slightly better results. Both algorithms had pros and cons, but NormFinder consistently provides a better reduction in sample variation across datasets.
Funding: OVC Scholarship, Pathobiology Grad Growth Stipend, OVC Pet Trust

---

2. Cardio-oncology: Premetastatic, therapy-naïve ovarian cancer causes cardiac dysfunction and inflammation

L.M. Ogilvie[1], B. Coyle-Asbil[2], K. Cochrane[2], B. Garlisi[1], J. Petrik[1], J.A. Simpson[2]* 1. Department of Biomedical Sciences, Ontario Veterinary College; 2. Department of Human Health and Nutritional Sciences, College of Biological Science, University of Guelph.

Introduction: Cardio-oncology research focuses on how cancer treatment influences cardiac health. Indeed, many cancer therapeutics exert cardiotoxic effects. Studies also demonstrate that advanced, therapy-naïve cancer causes cardiac atrophy and dysfunction. However, the underlying causes of cardiac impairments before metastasis or cachexia have not been investigated. Thus, we assessed cardiac structure, function, and molecular signalling in premetastatic epithelial ovarian cancer (EOC).
Methods: We used an orthotopic, syngeneic mouse model of EOC. Transformed ovarian surface epithelial cells from C57BL/6 mice (ID8; 1.0x106) were injected under the ovarian bursa. In this model, 60 days post-tumour induction, mice develop large ovarian masses, numerous peritoneal

lesions, and abdominal ascites, consistent with clinical features of stage III (advanced) EOC. To determine whether cardiac abnormalities precede advanced malignancy, at 45 days post-tumour induction, left ventricular (LV) function was assessed by hemodynamics. Histology quantified cardiomyocyte size and capillary density. RNA sequencing was performed on LV tissue to determine differential gene expression and upregulated pathways in EOC compared to shams. NLRP3 inflammasome activation in EOC was measured by RT-qPCR and western blot.
Results: In the absence of observable metastasis, tumour-bearing mice presented with systolic and diastolic dysfunction with no evidence muscle atrophy. Myocardial capillary density decreased with EOC, which was associated with hypoxia, pro-inflammatory signalling, and NLRP3 inflammasome activation.
Conclusion: We demonstrate that premetastatic, therapy-naïve EOC causes cardiac dysfunction, pathological remodelling, and the upregulation of inflammation. These findings suggest that even in cancer patients diagnosed with early-stage disease, cardiac assessments may be an important component of patient management.
Funding: Natural Sciences and Engineering Research Council of Canada, Canadian Institutes of Health Research

---

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

Hayley Smith[1], Hayley Lau[1], Begüm Alural[1], Manali Tilak[1], Ben Staples[1], Laura New[1], Kévin Jacquet[2], Claire E. Martin[3], Anne-Claude Gingras[3], Nicolas Bisson[2], Jasmin Lalonde[1], Nina Jones*[1]
1. Department of Molecular and Cellular Biology, University of Guelph; 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; 3. Lunenfeld-Tanenbaum Research Institute, Sinai Health System, University of Toronto

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

---

4. 6-Methoxydihydroavicine (6ME) targets acute myeloid leukemia involving peroxisome proliferation-activated receptor delta (PPARδ).

Yingying Yang[1], Nikolina Vrdoljak[1], Ekaterina Parfenova[1], Jessica Luc[2], Paul A. Spagnuolo[1]. 1. Department of Food Science, University of Guelph; 2. Department of Biology, University of Waterloo.

Background: Acute myeloid leukemia (AML) is a devastating hematological malignancy with limited therapeutic options and poor survival outcomes. Therefore, the development of novel and selective anti-AML therapies is crucial. Our group recently identified 6-methoxydihydroavicine (6ME), a benzophenanthridine alkaloid commonly found in the Papaveraceae family, as a novel and selective anti-AML drug. Thus, this study explored how 6ME exerts its anti-AML activity.

Methods: AML cell lines and patient-derived cells were used to assess the cytotoxicity of 6ME in vitro and in vivo. Computational methods, immunoblotting, and co-IP-UHPLC analysis were used to predict and confirm that 6ME targeted PPARδ, a transcription factor involved in fatty acid oxidation (FAO). Meanwhile, high resolution respirometry evaluated cellular oxygen consumption rates to determine how 6ME affected mitochondrial metabolism and FAO.

Results: 6ME induced cytotoxicity in AML cell lines and patient-derived cells while sparing normal hematopoietic cells. Specifically, 6ME (IC50: 1.0± 0.13 μM) suppressed clonogenic growth of patient-derived AML cells with no effect on normal hematopoietic cells. Mouse engraftment studies showed that 6ME (5 mg/kg, three times/week for 4 weeks) reduced patient-derived AML cell engraftment in mouse bone marrow without imparting toxicity. Mechanistically, 6ME bound to PPARδ and significantly reduced its expression, as measured by co-IP UHPLC and immunoblotting, respectively. Further, 6ME reduced FAO-supported mitochondrial respiration in AML cells, which is consistent with reduced PPARδ expression.

Conclusion: Taken together, 6ME is a novel and selective anti-AML molecule that inhibits PPARδ.

Funding: University of Guelph

---

5. Glowing insights: Exploring ICG-NIR dosage variances for human and canine tumours

R.H. Gamage[1], A.S. Ram[1], C. McKenna[2], M. Oblak[2]*. 1. Department of Biomedical Science, and 2. Department of Clinical Studies, Ontario Veterinary College, University of Guelph.

Indocyanine green (ICG) is a fluorescence dye that when combined with specialized near-infrared (NIR) cameras will fluoresce (or glow-in-the-dark). The combination of intravenous ICG and NIR is frequently utilized in various medical procedures such as tumour imaging, lymph node napping and angiography. In addition, it has an extremely high safety profile. Although ICG-NIR is extensively utilized in human medicine for lymphatic mapping and tumour imaging, its use in veterinary oncology is growing. Through a comprehensive review of published studies using ICG-NIR for soft tissue sarcomas, lung tumours, hepatocellular carcinoma, breast cancer, thoracic duct and insulinoma cancers, we aimed to elucidate potential differences and similarities in ICG dosage between human and canine patients for tumour imaging applications. Factors such as tumour-type species-specific physiological differences were considered. There was some variation in the mean ICG dosage given to canine patients compared to humans and between tumour types. Canine dosages (0.05-5 mg/kg) are generally higher than human dosages (0.2-2.5 mg/kg. Hepatocellular tumours and insulinoma have similar ICG dosages between the two species. The dosage of ICG for lung cancer surgery was the highest compared to other tumour types in both species. By comparing dosage strategies between the two species across various tumour types, insights can be gained regarding the two-way translation of ICG-NIR and potential new applications in veterinary medicine.

Keywords: Indocyanine green, tumour imaging, comparative study, humans, dogs, oncology, ICG, dosage comparison, canine, retrospective study, NIR, SLN

---

6. Preclinical cat study: a step towards a translational oral tumour theragnostic.

Charly J. McKenna[1], Michael S. Valic[2], Mark Zhang[2], Sharon Tzelnick[2], Jason Townsend[2], Harley Chan[2], Esmat Najjar[2], Jonathan C. Irish[2], Michelle Oblak[1], and Gang Zheng[2]. 1. Ontario Veterinary College, University of Guelph; 2. Princess Margaret Cancer Centre, UHN, Toronto, ON.

Oral tumours are common in cats with the majority (>60%) being malignant oral cavity squamous cell carcinoma (OCSCC). While surgery is the gold standard, OCSCC presents challenges due to postoperative complications and an inability to achieve complete surgical margins, resulting in high rates of reoccurrence (>38%). Adjuvant therapies are ineffective or poorly tolerated. Spontaneous companion animal OCSCC tumours share several similarities to human subtypes, including clinical presentation, tumour biology and treatment strategies. In addition, the treatment challenges and concerns for local reoccurrence are shared.

The use of a multimodal nanoparticle comprised of porphyrin-lipids, porphysomes (pPS), may address the challenges of treating patients with OCSCC. The safety and utility of pPS and its

application for photodynamic therapy (PDT), fluorescence-guided surgery or both has been demonstrated in mice, rats, pigs, dogs and non-human primates. A 3+3 dose escalation safety study was completed to evaluate the safety threshold of a single intravenous (IV) pPs dose in 9 adult, healthy male purpose-bred cats. Cats were randomly assigned into dose groups (3-10mg/kg) and IV LRS-pPs delivered over a 1-hour period. Repeat blood collection and monitoring was completed for at least 1-month following pPs administration. No abnormal clinical or toxicological findings were observed. Changes in hematology, serum biochemistry, and urinalysis parameters, if detected, were transient within the first 24 hours of pPs administration and returned to baseline levels and/or within normal limits. The no observable effect level of a single IV pPs administration in cats is 10 mg/kg BW. Survival trials in cats have proven useful for translational cancer research and novel therapy development. Future applications of pPs will include client-owned cats with OCSCC.

Funding: Canadian Cancer Society

---

7. The association between circulating segmented neutrophil count and inflammatory markers in canine appendicular osteosarcoma

Chau, A.[1], Hillis, N.[1], Huntley, V.[1], Speare, R.[2], Schott, C.R.[2], Wood, R.D.[2], Mutsaers, A.J.[1], Wood, G.A.[2], Viloria-Petit, A[1]* 1. Department of Biomedical Sciences, and 2. Department of Pathobiology, Ontario Veterinary College, University of Guelph

Osteosarcoma (OSA) accounts for 80-90% of all malignant bone tumours in canines. Inflammation is a hallmark of cancer, and neutrophils are key mediators of both acute and chronic inflammation. Inflammatory molecules, such as C-reactive protein (CRP) and cytokines, mediate the recruitment of neutrophils to inflammatory sites. We previously observed that a high count of circulating segmented neutrophils (SN) correlated with reduced time to metastasis and overall survival in canine OSA patients. Independently, we found similar associations for several circulating inflammatory markers. Here, we investigated whether the levels of inflammatory markers correlated with the levels of circulating SN. Blood collected from canine OSA patients prior to amputation was used for CBC count and serum/plasma banking. Forty patients met the inclusion criteria, and had SN counts and concentrations recorded for interleukin-6 (IL-6), IL-8, interferon-γ-induced protein 10 (IP-10), granulocyte-macrophage colony-stimulating factor (GM-CSF), keratinocyte chemotactic-like (KC-like), and monocyte chemoattractant protein-1 (MCP-1). An ELISA was used to determine CRP concentration in the serum of 16 patients. A Spearman correlation analysis revealed minimal associations between SN count and any of the inflammatory markers: CRP ($r = 0.3736$), IL-6 ($r = –0.2757$), IL-8 ($r = 0.09199$), IP-10 ($r = –0.06022$), GM-CSF ($r = 0.02543$), KC-like ($r = –0.04718$), and MCP-1 ($r = –0.1545$) with $p>0.05$ in all analyses. The negative correlation trend for IL-6, IP-10, KC-like, and MCP-1 may suggest SN are exiting

circulation to enter tissue. Investigating the association between neutrophils and cytokines in OSA tissue is important to gain insights for future mechanistic studies.
Funding: OVC Pet Trust and NSERC

---

8. Use of synthetic protein secreting CHO cells for protein purification of Fc3TSR in the treatment of advanced stage ovarian and pancreatic cancer

Aitken, C., Garlisi, B., Lauks, S., & Petrik, J.*. Department of Biomedical Science, Ontario Veterinary College, University of Guelph

Ovarian and pancreatic cancer have become two of the most lethal cancers worldwide. Targeted therapies that inhibit signal transduction pathways have been advancing in cancer research to increase systemic therapy options. A major target of interest is angiogenesis, in which the tumor initiates a program that stimulates new blood vessel formation to meet the tumors metabolic needs. To target this pathway, we have developed a fusion protein (Fc3TSR) which is derived from the potent anti-angiogenic factor thrombospondin-1. Fc3TSR has multi-modal effects against tumor progression and has shown promise in both pancreatic and ovarian murine models. In this project, we seek to expand the use of Fc3TSR using genetically manufactured Chinese Hamster Ovarian (CHO) cells which release the protein into its microenvironment. This project aims to study these cells and their target protein secreting capabilities *in vitro*, and the ability to purify Fc3TSR for scale up experiments *in vivo*. We used genetically manufactured CHO cells with an inserted coding sequence for Fc3TSR, cultured them, and purified Fc3TSR from collected media. We sought to compare this purified Fc3TSR (F3R), with our original manufactured protein in our *in vivo* models of advanced stage pancreatic and ovarian cancer. The preliminary data of purified F3R from the modified cell line indicates a potential increase in potency of Fc3TSR derived from cell secretions, compared to systemic injection of the protein. If F3R provides an increased therapeutic effect, this opens opportunities for the use of manufactured cells in both the production and in cell therapy applications.
Funding: OVC, University of Guelph

---

9. Pseudolaric acid B targets CD147 to selectively kill acute myeloid leukemia.

Sheng Zou, Ekaterina Parfenova, Nikolina Vrdoljak, and Paul A. Spagnuolo. Department of Food Science, University of Guelph.

Acute myeloid leukemia (AML) is an aggressive cancer of the blood and bone marrow. Outdated treatment regimens require improvement to overcome the current low rates of patient survival. Overexpression of CD147, a transmembrane glycoprotein, enables cancer cell survival. A recent proteomics study revealed that a plant-derived bioactive, pseudolaric acid B (PAB), targets

CD147 in human cancer cells. However, the anti-leukemic effects and detailed mechanism of PAB in AML are unknown.

The aim of this study was to determine the effects of PAB in AML. Cell lines and patient-derived AML cells were used to investigate the anti-leukemic effects of PAB. CD147 gene-specific short hairpin RNA (shRNA) knockdown, proliferation assay, flow cytometry, and immunoblotting were used to confirm the role of CD147 in AML and to assess the detailed downstream mechanisms of PAB-induced leukemia cell death. PAB induced selective cytotoxicity in AML cell lines and patient-derived cells (IC50: 1.59 ± 0.47 μM); there was no effect on normal peripheral blood stem cells. Knockdown of CD147 in AML cells significantly suppressed cell growth and proliferation and decreased NF-κB and anti-apoptotic Bcl-2 expression, indicative of effects on downstream CD147 pathways. PAB also suppressed NF-κB and anti-apoptotic Bcl-2 proteins, CD147's downstream targets, to induce apoptosis in AML cells. Overall, these results identified PAB as a potential plant-derived, novel therapeutic for AML by targeting the transmembrane glycoprotein CD147.

Funding: University of Guelph, The Leukemia & Lymphoma Society of Canada

---

10. Modulation of pyruvate kinase M2 activity for the treatment of acute myeloid leukemia.

Nikolina Vrdoljak, Ekaterina Parfenova, Paul A. Spagnuolo. Department of Food Science, University of Guelph.

Acute myeloid leukemia (AML) is an aggressive malignancy of the blood and bone marrow, in dire need of new therapeutic options to improve patient outcomes. Pyruvate kinase M2 (PKM2) is an isoform of pyruvate kinase (PK) that is highly expressed in malignant cells that leads to a reduction in PK activity and impaired cancer cell metabolism. Previous work has shown that modulation of PKM2 can target this abnormal cancer cell metabolism, thereby making PKM2 a promising target for AML therapy.

In this study, the functional importance of PKM2 in AML cell proliferation and metabolism was explored. Lentiviral-mediated knockdown of PKM2 (shPKM2) decreased cell proliferation and colony formation, and in pre-clinical mouse models led to reduced leukemia cell bone-marrow repopulation and extended mouse survival. Mechanistically, isotope tracing for glucose flux analysis demonstrated impaired glucose metabolism in shPKM2 cells. A high throughput screen identified a small molecule modulator of PKM2 activity that leads to specific reduction of AML cell viability and colony formation. High resolution respirometry also indicates an impairment of metabolism. Taken together, modulation of PKM2 activity plays a role in AML metabolism, and modulating PKM2 activity may be an effective therapeutic mechanism for the disease.

Funding: University of Guelph, Leukemia & Lymphoma Society of Canada

---

11. Is cytomorphology predictive of immunophenotype of chronic lymphocytic leukemia in dogs?

K. Morrison, G. Diamantino, J. Mori, E. Stacey, D. Bienzle*. Department of Pathobiology, Ontario Veterinary College, University of Guelph.

Background: Canine chronic lymphocytic leukemia (CLL) is a neoplasm of mature lymphocytes of T- or B-cell type. It is the most commonly diagnosed leukemia in dogs and is often found incidentally in middle aged to older patients where lymphocytosis is noted on a CBC. Diagnosis is typically based on the finding of persistent lymphocytosis, a characteristic cytomorphology and immunophenotyping by flow cytometry. Previous studies have shown that the majority of CLLs are of T-cell type and comprised of CD8+ lymphocytes with a granular cytomorphology. Objective: The aim of this study was to determine the accuracy with which immunophenotype, hematocrit, and lymphocyte count could be predicted in cases of canine CLL based on cytologic assessment. Methods: Blood smears from cases of chronic lymphocytic leukemia submitted for flow cytometric immunophenotyping were reviewed by 4 clinical pathology residents and one board certified clinical pathologist. Slides were evaluated for severity of anemia (HCT <30, 30-40 or >40 L/L), lymphocyte count (<10, 10-30, >30 x109/L) and predicted immunophenotype (T-CLL vs B-CLL). Correlations and interobserver agreement were tested by Spearman correlation coefficient and Fleiss' kappa, respectively.
Results: Of 66 cases of CLL, 36 cases (55%) were T- and 30 cases (45%) were B-CLL. Of the T-CLL cases, 58% were CD8+ and 71% of these cases had granular cytomorphology. Correlation between rater prediction and immunophenotype ranged from very weak to strong with fair interobserver agreement (□=0.34). For lymphocyte count there was strong correlation between rater scores and the true count, and fair interobserver agreement (□=0.39). There was overall weak to moderate correlation between rater scores and the true hematocrit, and only slight interobserver agreement (□=0.03).
Conclusion: While assessment of cytomorphology has a critical role in the diagnosis of CLL, ability to predict immunophenotype is limited.

---

12. Analysis of SNARE phosphorylation during cell-ECM interactions

E. Perehiniak, K. Williams, M. Coppolino*. Department of Molecular and Cellular Biology, University of Guelph.

SNAREs (Soluble N-ethylmaleimide-sensitive factor Attachment Protein Receptors) are proteins that mediate membrane fusion during vesicle trafficking pathways in cells. SNAREs have well-defined roles in neurotransmission and hormone secretion, and have recently been identified to play important roles in cell-extracellular matrix (ECM) interactions, including cell adhesion, cell

migration, and cell invasion. SNARE-mediated trafficking is responsible for the transport of cellular components to and from ECM interaction sites. Components such as MT1-MMP, a key proteolytic enzyme that facilitates ECM degradation in invasive tumour cells, has been shown to be trafficked in a SNARE-dependent manner. This suggests that the regulation of SNAREs could play an important role in the intracellular trafficking that contributes to tumour cell invasion. Phosphorylation is a key post-translational modification, but its role in SNARE-mediated cell-ECM interactions and metastasis are largely unknown. Preliminary work suggests phosphorylation of SNAREs is a key mechanism that can regulate cell-ECM interactions such as adhesion and migration. A SNARE complex containing Stx4-SNAP23-VAMP7 that plays a previously characterized role in cell-ECM interactions appears to be regulated by phosphorylation. Particularly, Stx4 and SNAP23 phosphorylation decreases during ECM degradation which is associated with increased SNARE interaction in tumour cells. These findings will be further explored by employing site-directed mutagenesis to investigate the impact of phosphorylation at specific amino acid residues in SNAREs. Current and future research aims to elucidate the role of SNARE regulation by phosphorylation in the trafficking of cellular components that are essential to cell-ECM interactions and cell invasion.
Funding: NSERC

---

13. Fc3TSR directed remodeling of the tumour microenvironment to enhance efficacy of immunotherapies and immune cell migration in a murine model of pancreatic ductal adenocarcinoma

B. Garlisi[1], C. Aitken[1], S. Lauks[1], J. Lawler[2], J. Petrik[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 with late diagnosis and distant metastasis. Angiogenesis, vessels sprouting off pre-existing vasculature, is critical for tumour growth and metastasis, delivering necessities to tumours. In cancer, there is an upregulation of angiogenic promoters, increasing the rate of vessel development, creating leaky vessels and poor perfusion. Fc3TSR, designed from the potent angiogenic inhibitor thrombospondin 1, has normalized the tumor microenvironment (TME) in ovarian cancer in our lab), and we hypothesize it can enhance therapy uptake and efficacy in PDAC.
Methods: Using our orthotopic syngeneic murine model of PDAC, we injected 2.5x104 PDAC cells into the pancreas of mice. Administration of Fc3TSR (0.158mg/kg) or PBS occurred on day 14 and 21. Immune checkpoint inhibitor (ICI) (25ug) injections were administered on day 23 and 26. Mice were euthanized on day 30, metastatic lesions were counted, and tumours were collected. Tumors were stained for apoptotic, proliferative and vasculature markers, hypoxia, and immune cells.

Results: Fc3TSR, alone and with ICIs, significantly reduced tumour weight and metastasis compared to PBS. Fc3TSR significantly remodeled the TME compared to PBS. Fc3TSR significantly increased CD4 and CD11c in tumours, but the combination of Fc3TSR + ICIs increased this further compared to either alone. Staining for apoptotic, lymphatic vessels and other immune cells, and staining of lymph nodes are currently being analyzed.
Conclusion: This data could provide evidence of the importance of normalizing the TME before cancer therapies to ensure effective delivery and strong immune responses in PDAC patients.
Funding: University of Guelph, Cancer Research Society, Canadian Institutes of Health Research, Health Canada

---

14. Elucidating interaction partners of Gasdermin E (GSDME) post-cleavage during pyroptosis.

Kaslyn Kallio, Noah Phippen, Jordon Inkol, Sam Workenhe. Department of Pathobiology, University of Guelph.

Pyroptosis is an immune-stimulatory cell death that can be induced by cytotoxic anticancer therapies. The cleavage of gasdermins into N-terminal and C-terminal fragments are essential to the execution of pyroptosis. The N-terminal fragment inserts into the plasma membrane to form pores, leading to lysis and release of inflammatory mediators. Studies have investigated the plasma membrane rupture mechanism of Gasdermin D (GSDMD), but it is uncertain how much crossover there between GSDMD and Gasdermin E (GSDME). Both how the N-terminal fragment of Gasdermin E (GSDME-N) localizes to and oligomerizes in the plasma membrane and the role of the C-terminal fragment (GSDME-C) other than autoinhibition is unknown. The goal of this research is to identify new interaction partners of GSDME-N and GSDME-C in cancerous and non-cancerous conditions to further elucidate the mechanism of cell lysis during pyroptosis. We used the proximity-dependent biotinylation approach, BioID, to investigate these questions. We developed inducible GSDME-N/C cell lines tagged with a biotin ligase. Upon expression of GSDME-N/C and the addition of biotin, proteins within proximity of the gasdermin proteins will be biotinylated. They are then pulled down via streptavidin purification and analysed by mass spectrometry. Based on the protein candidates from the mass spectrometry analysis, direct interactions of the gasdermin fragments will be determined using co- and western blotting. The results of these studies will further explore the mechanism of pyroptosis and identify differences in cancerous and non-cancerous cells undergoing pyroptosis.

---

15. Nck adaptor proteins regulate breast cancer cell metastasis and invasion.

Erka Shata[1], Adam Golding[1], Sarah Wismer[1], Una Adamcic-Bistrivoda[1], Claire Martin[1], Laura New[1], Peihua Lu[1], Manali Tilak[1], Megan Brasher[1], Ji Shi[2], Hongmei Zhao[3], Caihong Ma[4], Marc Coppolino[1], Josie Ursini-Siegel[5], and Nina Jones[1]*. 1. Department of Molecular and Cellular Biology, University of Guelph; 2. Department of Gastrointestinal Surgery, and 3. Department of General Surgery, and 4. Center for Human Reproductive Medicine, Peking University Third Hospital, Beijing, China; 5. Lady Davis Institute for Medical Research, McGill University.

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 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.

Funding: CIHR-IRSC; Cancer Research Society

---

16. Differential regulation of the KEAP1-NRF2 antioxidant pathway in long and short-lived bird species.

Thompson Worden, Morla Phan, Geoffrey A. Wood*. Department of Pathobiology, University of Guelph

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 lifespans 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 the 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.
Funding: NSERC

---

17. Pseudogene contribution to copy number variation and cancer risk.

M. Phan[1], T. Worden[1], S. Adamowicz[2], S. Lair[3], M. Seguel[1], C. Jardine[1], G. A. Wood[1]*. 1. Department of Pathobiology, Ontario Veterinary College, and 2. Department of Integrative Biology, University of Guelph; 3. Département de sciences cliniques, Faculté de médecine vétérinaire, Université de Montréal.

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.
Funding: NSERC

---

18. From lab to leash and bench to bedside: transforming cancer care for pets and people.
C. McKenna[1], J. Petrik[2], M.L. Oblak[1]. 1. Department of Biomedical Sciences, University of Guelph; 2. Department of Clinical Studies, University of Guelph

Today, 238 Canadians died of cancer, underscoring the urgent need for advancements in therapy development. Despite significant progress in preclinical research, including genomics and human patient-derived xenograft models, the methodology for drug discovery has remained largely unchanged for almost five decades. This stagnation in innovation is reflected in the lack of confidence in our therapy development pipeline. Cancer is the leading cause of death in both pets and humans, with thousands diagnosed annually in Canada alone, a significant portion succumbing to the disease. In response to this dual crisis, our interdisciplinary team of basic scientists, veterinary oncologists, and human oncologists proposes a groundbreaking approach: a "Bench to Bedside" therapy development pipeline. This innovative pipeline integrates all stakeholders from the outset and harnesses the predictive power of companion animals (CAs; cats and dogs) with naturally-occurring cancers as translational models.
Companion animals not only share our homes but also share many diseases and disorders with humans, spanning from cancers to cardiac diseases to psychiatric disorders. Furthermore, our pets exhibit immense genetic diversity and inhabit a shared, intricate environment with humans. Consequently, naturally occurring diseases in CAs offer an almost perfect model—and a vastly underutilized opportunity—for pioneering new disease treatments. Our multi-species translational cancer research program seeks to capitalize on the unique insights garnered from studying companion animal cancer patients. By establishing a Bench to Bedside approach, we aim to seamlessly transition novel discoveries from in vitro experimentation to preclinical models, companion animal cancer patients, and ultimately, human cancer patients.

---

19. Verteporfin photodynamic therapy induces cellular features of immunogenic cell death in metastatic murine osteosarcoma cells
A. Dhillon[1], E. Pepeljak[1], S. Workenhe[2], J. Hummel[3], A. Viloria-Petit[1]* 1. Department of Biomedical Sciences, and 2. Department of Pathobiology, Ontario Veterinary College, University of Guelph; 3. Illumacell, ON, Canada

Osteosarcoma (OS) is the most common primary bone cancer in both human and dogs, and metastatic disease is the main cause of death by OS in both species. This outcome has not changed for the last 4 decades, making it imperative to find alternative therapies. Verteporfin (VP)-photodynamic therapy (PDT), approved to treat macular degeneration in humans, holds promise. VP has been shown to inhibit the growth of several cancer types in the absence of light-induced activation. Cancer-targeting properties of VP have been attributed in part to its capacity to inhibit the transcriptional co-activator yes-associated protein (YAP). Thus, we and others have provided evidence of the role of YAP in canine and human OS progression. Our data points to VP as a drug that targets OS metastasis by influencing both tumour cells and their immune microenvironment, which we hypothesize is by inducing immunogenic cell death (ICD), resulting in the activation of an anti-tumour immune response via exposure of damage associated molecular patterns (DAMPs) by the dying tumour cells. To address this possibility, we treated the aggressive murine osteosarcoma cell line K7M2 with YAP targeting and non-targeting doses of VP, both in the absence and presence of LED red light (Illumacell, ON, Canada). We employed immunofluorescence, immunoblotting and a DNA fragmentation test to assess the expression of YAP, DAMPs, and cell death following treatment. The data indicates that effects on YAP expression are modulated by VP concentration and light, and that light-induced activation is required for cell death and DAMP exposure. Ongoing studies aim to test the capacity of VP-PDT cells to activate an anti-tumour immune response, and to target metastatic disease in murine syngeneic surgical models of osteosarcoma employing K7M2 and other cell lines.
Funding: Mitacs, OVC Pet Trust, NSERC.

---

20. Hindering of osteosarcoma lung metastasis by verteporfin associates with changes in the tumour microenvironment.

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

Osteosarcoma (OS) is the most common bone cancer. Death by OS is primarily due to pulmonary metastasis, occurring in 15% to 40% of patients both pre- and post-treatment. Therefore, there is a need for improved therapies. Both, Yes-associated protein 1 (YAP1) and autophagy drive OS metastasis and drug resistance. Verteporfin, a photosensitizer approved for photodynamic therapy of macular degeneration, targets YAP1 and the autophagy mediator p62, by causing the formation of covalently crosslinked oligomers of these proteins. Based on this, we hypothesize verteporfin will impede cancer cell thriving under stress, such as during the metastasis process. Therefore, we assessed verteporfin's effect on OS lung colonization and the lung microenvironment, specifically innate immune cell infiltration and biomechanical properties.

BALB/c mice were tail vein injected with K7M2 osteosarcoma cells or PBS. The K7M2 injected animals were randomly divided to either receive verteporfin (20 mg/kg) or vehicle intraperitoneally twice a week. Lung samples were collected on days 4, 8, 16, and 24 for histological, biomechanical, and flow cytometry analysis. Verteporfin significantly reduced the metastatic burden by day 24, and this paralleled a small yet significant increase in lung stiffness, and a reduction in both innate immune cell infiltration of the lung and the expression of YAP target genes. No changes were observed in collagen deposition in metastatic nodules. However, mass spectrometry analysis identified higher fibronectin levels in verteporfin-treated lung tissue. Our findings suggest that verteporfin has anti-metastatic properties and targets the tumour microenvironment of the lung, warranting further investigation in the context of OS therapy.
Funding: Mitacs, NSERC, OVC Pet Trust.

---

21. The Ontario Veterinary College Veterinary Biobank: facilitating translational research.

Deirdre Stuart[1], Latasha Ludwig[2], Charly McKenna[1], Brenda Coomber[3], Paul Woods[3], Michelle Oblak[1], Geoffrey Wood[2]. 1. Department of Clinical Studies, and 2. Department of Pathobiology, and 3. Professors Emerita/us, Ontario Veterinary College, University of Guelph.

The Ontario Veterinary College (OVC) Veterinary Biobank (OVCVB) facilitates basic and translational veterinary research. Located in the OVC Mona Campbell Animal Cancer Centre, the OVCVB is the first veterinary biobank in Canada. To ensure that the biobank follows best practices, it is registered with the Canadian Tissue Repository Network (CTRNet) and International Society for Biological and Environmental Repositories (ISBER), and looks forward to its upcoming membership in the newly developed federation of Ontario biobanks including the Ontario Tumour Bank (OTB). With a current repository of over 1,850 cases, samples collected and stored include serum, plasma, buffy coat, urine, and tissue. While the primary focus to date has been oncology samples, the scope of the biobank is expanding to include other diseases and areas of interest. Tissue samples are collected immediately following surgical excision and stored as flash-frozen, RNAlater- and CryoMatrix-preserved. Tumour tissue is also formalin-fixed and analyzed by a pathologist for quality control. A wide variety of neoplasms have been collected: the most prevalent in dogs are soft tissue tumour (STT), lymphoma and osteosarcoma; and in cats, STT, mammary carcinoma and osteosarcoma. There are also 12 cell lines from primary tumours, with more in development. The OVCVB 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 OVCVB have been used in 36 intramural and extramural research projects to date. The OVCVB is a unique resource with the mission to facilitate basic, comparative, and translational research to improve the lives of companion animals. In addition,

data from spontaneous companion animal disease can complement preclinical rodent studies, with the augmented potential to contribute to comparative human research.

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