

8th Annual Cancer Research Symposium Program

**Wednesday, May 27, 2015
Room 1714 LLC, Ontario Veterinary College
University of Guelph**

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Introductory Remarks

Welcome to the 8th Annual Guelph ICCI Cancer Symposium. This meeting is intended to bring together individuals interested in the study of any aspect of cancer in any species, from the most basic elements, to clinical therapies and on to social, emotional and ethical aspects of this often-devastating disease. Again this year we are highlighting local, national and international researchers. This year in particular we have several presentations about novel cancer therapies.

Through interactions facilitated by this meeting, it is hoped that new insights and collaborations will develop that will enhance the research and scholarship in the area of cancer research at the University of Guelph and collaborating institutions. We would like to thank the OVC Dean's Office and the Arthur Willis Visiting Professorship for financial support of the meeting, and for sponsoring the visit of Dr. Nicola Mason, who is this year's Arthur Willis Distinguished Speaker. We hope you will find this symposium interesting and informative, and that it leads to fruitful research collaborations for all our attendees.

Co-Organizers

Tony Mutsaers and Brenda Coomber

Clinical Studies and Biomedical Sciences, University of Guelph



Thanks to Daphne Summers, OVC Office of the Dean, for her administrative expertise and invaluable assistance in organizing this event, to David Wood, OVC IT for help with on line activities, and to Adrian Hollingbury and his crew at the OVC Dining Hall for help with set up and refreshments. The research projects presented here and the trainees performing these studies were collectively supported by grants, scholarships and contracts from: The Canadian Cancer Society; The Cancer Research Society; Leukemia & Lymphoma Society of Canada, Canadian Hematology Society; The Banting Research Foundation; Canada Stem Cell Network; Roswell Park Alliance Foundation; Sao Paulo Research Foundation; CIHR; NSERC; US Army Department of Defense; OGS; CIHR Vanier Canada Graduate Scholarship; University of Waterloo; OVC Pet Trust; Smiling Blue Skies Cancer Fund; OVC Bull Foundation; CAPES; Office of the Dean & Department of Clinical Studies, Ontario Veterinary College, University of Guelph.

ICCI 8th Annual Cancer Research Symposium

Wednesday May 27, 2015

Morning Session: Room 1714, OVC LLC

9:00 - 9:05 Welcome and Introductory Remarks

9:05 - 9:50 Guest Speaker

Dr. John Ebos, Assistant Professor of Oncology, Departments of Cancer Genetics & Medicine, Roswell Park Cancer Institute, Buffalo, NY, USA

Drug resistance in the era of stromal-targeted therapy: Lessons from angiogenesis inhibitors

9:50 - 10:05 short talk from submitted abstracts:

1) Ritesh Briah, Department of Biomedical Sciences, OVC, University of Guelph

Inhibition of Akt isoforms in NSCLC tumour suppression

10:05 - 10:35 Coffee Break and Poster Viewing Room 1707 B & C, OVC LLC

10:35 - 10:50 short talk from submitted abstracts:

2) Dr. Paul Spagnuolo, School of Pharmacy, University of Waterloo

Inhibition of fatty acid oxidation selectively eliminates acute myeloid leukemia cells and leukemia stem cells

10:50 - 11:05 short talk from submitted abstracts:

3) Stacey Butler, Department of Biomedical Sciences, OVC, University of Guelph

Characterization of human colorectal cancer stem cells

11:05 - 11:20 short talk from submitted abstracts:

4) Courtney Schott, Department of Pathobiology, OVC, University of Guelph

Evaluation of histologic features for predicting outcome in canine appendicular osteosarcoma

11:20 - 11:35 short talk from submitted abstracts:

5) Michelle Oblak, Department of Clinical Studies, OVC, University of Guelph

The importance of research collaboration in surgical oncology

11:35- 1:20 Room 1707 B & C, OVC LLC

Poster Session and Lunch (provided)

poster presenters please attend your posters between 12:00 and 1:00

Afternoon Session: Room 1714, OVC LLC

1:30 - 2:15 Guest Speaker

Dr. Yonghong Wan, Professor, Pathology & Molecular Medicine, McMaster Immunology Research Centre, Faculty of Health Sciences, McMaster University, Hamilton, ON

Oncolytic vaccine therapy of cancer

2:15 - 2:30 short talk from submitted abstracts:

6) Megan Strachen-Whaley, Department of Pathobiology, OVC, University of Guelph

Using Viruses to Potentiate Epigenetic Modifier-Mediated Killing of Leukemic Cells

2:30 - 2:55 Invited Local Speaker

Dr. Stefan Keller, Pathobiology, OVC

Diagnosing lymphoid neoplasia -Clonality testing in the era of high-throughput sequencing

2:55 - 3:10 short talk from submitted abstracts:

7) Nariman Deravi, Department of Pathobiology, OVC, University of Guelph

Prognostication of Canine T-Cell Lymphoma

3:10 - 3:25 short talk from submitted abstracts:

8) Portia Kalun, Department of Human Health and Nutritional Sciences, CBS, University of Guelph

Knockdown and knockout of the 1,25 D3-MARRS receptor inhibits monocytic differentiation of NB4 leukemia cells

3:25 - 3:30 break

3:30 - 4:30 Keynote Speaker

Dr. Nicola Mason, Associate Professor of Medicine, Departments of Clinical Studies & Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, USA

Targeted, Recombinant Listeria-based Immunotherapy in the Treatment of Osteosarcoma - David meets Goliath and the rest is history

4:30 - 5:15 Room 1707 B & C, OVC LLC

Closing Reception and final Poster Viewing

KEYNOTE PRESENTATION

3:30 OVC LLC Room 1714

Dr. Nicola Mason

BVetMed., Ph.D., Dipl. ACVIM

Associate Professor of Medicine, Departments of Clinical Studies & Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, USA

Targeted, Recombinant *Listeria*-based Immunotherapy in the Treatment of Osteosarcoma - David meets Goliath and the rest is history

Highly attenuated, recombinant *Listeria monocytogenes* can be used to safely deliver tumor antigens into antigen-presenting cells and induce potent anti-tumor immunity. Cytotoxic T cell responses induced by *L. monocytogenes* eliminate established tumors and prevent metastatic disease in multiple mouse models. However, the effectiveness of this approach in the setting of spontaneous disease is unknown. We have evaluated the ability of a recombinant chimeric human HER2/neu expressing *L. monocytogenes* as adjuvant therapy in the setting of minimal residual disease and in combination with radiation therapy in the setting of non-resectable disease to prevent metastases and delay primary tumor progression in dogs with spontaneous osteosarcoma. Promising results in both settings pave the way to USDA licensure for veterinary use and the initiation of adult and pediatric clinical trials in HER2+ neoplasia.

Dr. Mason graduated from the Royal Veterinary College, London and spent a year in private small animal practice. She performed a small animal internship at the University of Bristol and an Internal Medicine residency at the University of Pennsylvania after which she became a Diplomate of the American College of Veterinary Internal Medicine. She earned her PhD in Immunology from the University of Pennsylvania and performed her post-doctoral fellowship in the laboratory of Dr. Carl June at the University of Pennsylvania's School of Medicine. She joined the faculty of the Veterinary School at the University of Pennsylvania in 2006. Her research focuses on developing immune therapeutic approaches to effectively target cancer and prevent metastatic disease in companion dogs, with the ultimate goal of identifying and accelerating successful therapies into the human and veterinary clinics. Her lab is currently focused on two main therapeutic strategies; recombinant listeria-based technologies and chimeric antigen receptor T cells (CAR-T). Target canine neoplasias include osteosarcoma, lymphoma and hemangiosarcoma. She holds the Pamela Cole Chair in Companion Animal Medicine, is an Associate Director of the Mari Lowe Center for Comparative Oncology and serves as the Director of the PennVet Tumor Tissue Bank

Past ICCI Symposium Arthur Willis Distinguished Speakers

2014	Deborah Knapp	2011	Cheryl London
2013	David Argyle	2010	Matthew Breen
2012	Timothy Fan	2009	Barbara Kitchell

GUEST SPEAKER

9:10-9:55 OVC LLC Room 1714

Dr. John Ebos, PhD

Assistant Professor of Oncology, Departments of Cancer Genetics & Medicine at Roswell Park Cancer Institute (RPCI), and Department of Pharmacology and Therapeutics, State University of New York at Buffalo

Drug resistance in the era of stromal-targeted therapy: Lessons from angiogenesis inhibitors

One consistent (and often frustrating) aspect of cancer research is learning that a preclinical ‘breakthrough’ did not eventually lead to a new therapeutic benefit for patients. This may stem, at least in part, from the fact that new drugs are often tested in patients with advanced metastatic disease while mouse models typically examine only localized ‘primary’ tumors. Recent evidence suggests that this testing gap may explain the failure to predict some of the modest clinical benefits of approved antiangiogenic therapies, which are drugs that aim to block the growth of new blood vessels – a critical component of tumor growth and spread. In theory, an advantage of antiangiogenic therapy is that, by blocking blood flow, the tumor will starve and eventually shrink. Additionally, ‘normal’ blood vessel cells might be less resistant to treatment compared to tumor cells. Unfortunately, and despite the clinical success of antiangiogenic therapy, drug resistance still occurs and disease eventually returns. Currently, the origins of this failure remain unclear. Growing evidence suggests that antiangiogenic treatment can elicit ‘reactions’ from normal stromal cells that may assist in compensating for VEGF pathway blockade or, more provocatively, even promote ‘rebound’ regrowth when therapy is stopped. Current investigations in my laboratory aim to study antiangiogenic therapy resistance in mouse models that recapitulate the steps of metastasis, including initial primary tumor growth, surgery, and the generation of spontaneous distant disease. Our studies have uncovered evidence that contrasting effects of antiangiogenic therapy may explain why treatment can be less effective in preventing micrometastatic disease and reveal that concerted tumor and stroma ‘reactions’ to inhibition may yield reversible and irreversible effects on rebound growth when treatment is stopped.

Dr. Ebos completed his undergraduate training at McGill University and earned his PhD in the Department of Medical Biophysics at the University of Toronto under the mentorship of Dr. Robert S. Kerbel where he studied novel circulating molecules as potential surrogate markers of cancer progression and antiangiogenic drug efficacy. Dr. Ebos' doctoral studies led to the discovery of a soluble form of a key receptor for the vascular endothelial growth factor (VEGF) pathway, the ligand/receptor pairing that is the central target for all antiangiogenic therapies now FDA approved in several different cancer types. This soluble receptor (termed 'sVEGFR-2'), along with several other circulating cytokines, were then studied as indicators of off-target 'host' effects following therapy and investigated for their potential to facilitate disease progression. Dr. Ebos' laboratory currently focuses on the molecular mechanisms that drive intrinsic and acquired drug resistance to stromal inhibitors in the treatment of spontaneous metastatic disease in mouse surgical models. This includes late-stage systemic metastasis as well as early-stage perioperative settings, such as with adjuvant and neoadjuvant treatment settings. The long-term goal of these efforts is to conduct preclinical models in conjunction with ongoing clinical trials so that benefits can be more readily predicted and rational designs for subsequent/alternative therapies can be developed.

GUEST SPEAKER

1:15-2:00 OVC LLC Room 1714

Dr. Yonghong Wan, MD

Professor, Pathology & Molecular Medicine, McMaster Immunology Research Centre, Faculty of Health Sciences, McMaster University, Hamilton, ON

Oncolytic vaccine therapy of cancer

Recent clinical data has emphatically demonstrated the capacity of our immune systems to eradicate even advanced cancers. Oncolytic viruses (OVs) although originally designed to act as tumour lysing therapeutics have now been shown clinically to initiate systemic anti-tumour immune responses. Cell signalling pathways that are activated and promote the growth of tumour cells also favour the growth and replication of viruses within the cancer. The ability to engineer OVs to express immune stimulating cargo, their induction of immunogenic tumour cell death and the exquisite targeting of OVs to tumour beds argues that they are the ideal reagents to enhance anti-tumour immune responses. Coupling of OV therapy with tumour antigen vaccination, immune checkpoint inhibitors and adoptive cell therapy appear ready to converge towards a new generation of multimodal therapeutics to improve outcomes for cancer patients.

Dr. Yonghong Wan is a Professor in the Department of Pathology and Molecular Biology at McMaster University and a member of the ORBiT program and the BioCanRx NCE network. His research interests focus on dendritic cell immunobiology and cancer immunotherapy. His collaboration with Drs. John Bell, Brian Lichty and David Stojdl was instrumental in developing an oncolytic vaccine platform that is now in the early stages of a phase I clinical trial.

INVITED LOCAL SPEAKER:

Stefan M. Keller

Assistant Professor, Department of Pathobiology, OVC, University of Guelph

Diagnosing lymphoid neoplasia -Clonality testing in the era of high-throughput sequencing

A correct diagnosis is the basis for adequate treatment. Clonality testing is a molecular method to diagnose lymphoid neoplasia if testing by conventional means is equivocal. In veterinary medicine, the sensitivity of clonality assays has been insufficient because pertinent sequence data was unavailable at the time of assay development. Recent advances in sequencing technology and bioinformatics, however, are revolutionizing the field. Not only do the new data facilitate the design of assays with improved sensitivity. High-throughput sequencing will eventually result in a fundamental change in testing methodology, which will significantly augment test versatility and accuracy. This talk will give an introduction to the principles of clonality testing, review clonality testing in veterinary medicine and discuss the potential of new sequencing technologies for the diagnosis and monitoring of lymphoid neoplasms.

ORAL PRESENTATIONS SHORT TALKS FROM SUBMITTED ABSTRACTS: (*Study Leader)

Morning Session:

1) Ritesh Briah, S. E. Franks, R. A. Moorehead*

Department of Biomedical Sciences, OVC, University of Guelph

Inhibition of Akt isoforms in NSCLC tumour suppression

Lung cancer is the leading cause of cancer related deaths worldwide. In non-small cell lung cancer (NSCLC), signaling kinase Akt is found to be hyper-activated and is involved in tumorigenesis through regulating cell survival, proliferation, migration and metabolism. Currently pan-Akt (Akt1-3) inhibitors are being evaluated in clinical trials, however Akt isoforms have recently been shown to have different physiologic roles. To determine whether using a pan-Akt inhibitor is the best therapeutic strategy, we compared the effects of Akt1 (A-674563), Akt2 (CCT128930), and pan-Akt (MK-2206) inhibition on tumour suppression properties in A549 and NCI-H358 human cancer cell lines. Cell survival curves determined that the Akt2 inhibitor concentration needed to kill 50% of cells (IC₅₀) was 28 and 26 times greater than the Akt1 inhibitor in A549 and NCI-H358 cells respectively, with the IC₅₀ for the pan-Akt inhibitor being 4-10 times greater than the Akt1 inhibitor. Immuno-fluorescence quantification of phospho-histone H3 in A549 cells treated with similar doses of each inhibitor did not show a decrease in proliferation status, however quantification of Annexin V through flow cytometry indicated a significant increase in apoptosis only in cells treated with the Akt1 inhibitor. Similarly, a measure of cell migration using scratch wound assays demonstrated that Akt1 inhibition was the most effective at slowing cell motility. These results indicate that Akt1 inhibition is more effective at inhibiting survival and migration in human NSCLC cells than Akt2 inhibition and that Akt1 may be a more appropriate target for treatment of NSCLC cases than pan-Akt inhibition.

Supported by: CCSRI, CIHR, OGS

2) Eric A. Lee and Paul A. Spagnuolo

School of Pharmacy, University of Waterloo, Kitchener, ON

Inhibition of fatty acid oxidation selectively eliminates acute myeloid leukemia cells and leukemia stem cells

Acute myeloid leukemia (AML) is an aggressive malignant disease characterized by poor patient outcome and suboptimal front-line chemotherapy. To identify novel anti-AML compounds, we performed a high-throughput screen of a natural products library (n=800) and identified avocatin B as a potent and novel anti-leukemia agent. Avocatin B, at concentrations as high as 20µM, had no effect on normal peripheral blood stem cell viability. In contrast, it induced death of primary AML cells with an EC₅₀ of 1.5-5.0 µM. Selective toxicity towards a functionally defined subset of primitive leukemia cells was also demonstrated. Avocatin B (3µM) reduced the clonogenic growth of AML progenitor cells with no effect on clonogenic growth of normal hematopoietic stem cells. Further, treatment of primary AML cells with avocatin B (3µM) diminished their ability to engraft into the bone marrow of pre-conditioned, NOD/SCID mice (t₁₈=6.5; p<0.001). Mechanistically, we demonstrated that avocatin B accumulates in mitochondria and inhibits fatty acid oxidation resulting in reduced NAD, NADPH and glutathione leading to ROS-dependent

apoptosis. Together, these results highlight a novel AML-therapeutic strategy by which mitochondria are targeted to impair cellular metabolism leading directly to AML and leukemia stem cell death.

3) Stacey Butler, Nathan Farias, Brenda L. Coomber*

Department of Biomedical Sciences, OVC, University of Guelph

Characterization of human colorectal cancer stem cells

Treatment of advanced colorectal cancer (CRC) is impacted by tumour heterogeneity and the development of drug resistance. Cancer stem cells (CSCs) are a subpopulation of tumour cells believed to be responsible for tumour recurrence due to their ability to self-renew, differentiate and evade current treatments. We used serial colonosphere culture to enrich for CSCs from two human CRC cell lines, HCT116 and SW480. Limiting dilution analysis determined that CSC enrichment significantly increased colonosphere formation ability compared to their respective parental cell lines. Characterization of colorectal CSC markers was performed via RT-qPCR and flow cytometry to determine the expression of CSC markers. CSC enriched cell lines had higher gene expression of CD44, CD166, and EpCAM compared to parental cell lines. However, flow cytometry analysis revealed that EpCAM was uniformly expressed among cell lines. CD166 and CD133 were expressed in virtually all HCT116-P cells, but in fewer SW480-P cells. Expression of aldehyde dehydrogenase (ALDH) has also been documented as a CSC marker and may also play a functional role in detoxification and chemotherapy resistance. The expression of several ALDH and ABC transporter family members was analyzed via RT-qPCR. Several of these genes were highly expressed in CSC enriched cell lines compared to parental cells. However, ALDH1A3 and ALDH3A1 were poorly expressed in SW480-CSCs compared to SW480-P cells. These results suggest we successfully enriched for CSCs from established cell lines despite the already high intrinsic CSC population in HCT116. The CSC markers examined were not uniformly expressed, supporting the need for reliable and universal markers to identify the CSC population in CRC.

Supported by: NSERC, OGS, OVC Dean's Scholarship

4) Courtney R. Schott, R.A. Foster, and G.A. Wood*

Department of Pathobiology, OVC, University of Guelph

Evaluation of histologic features for predicting outcome in canine appendicular osteosarcoma

All dogs diagnosed with osteosarcoma of the appendicular skeleton are given a poor prognosis for long term survival. Median survival time is longer when adjuvant chemotherapy is administered. There are two published canine osteosarcoma grading schemes that use a combination of histologic features: pleomorphism, mitoses, tumour matrix, necrosis and cellularity, but neither considered the effect of anatomic site or treatment. The objective of the current study was to determine the prognostic value of these grading schemes in a cohort of 85 dogs who underwent limb amputation plus adjuvant chemotherapy. Overall, the median survival time, as calculated by the Kaplan-Meier survival function, was 294 days (95% CI: 217-360, range 78-1458). Based on one grading system there were 11 grade I, 57 grade II, and 17 grade III tumours. For the other, there were 32 grade I, 34 grade II, and 19 grade III tumours. The median survival times for grades I, II, and III for each system were 385, 260, and 411 days, and 301, 260, and 282 days, respectively. Histologic grading of these cases (using either scheme) was not

significantly correlated with survival time. Furthermore, no individual histologic features (grading criteria) were identified as independent predictors of outcome when analyzed using the Cox Proportional Hazards model ($p=0.05$). These results show that histologic features (pleomorphism, mitoses, tumour matrix, necrosis and cellularity) have relatively little value in predicting survival time after standard of care therapy in canine appendicular osteosarcoma and that molecular markers should be investigated to improve prognostication. Supported by: OVC Pet Trust

5) Michelle Oblak

Department of Clinical Studies, OVC, University of Guelph

The importance of research collaboration in surgical oncology

Cancer plays an important role in a number of research projects both clinical and non-clinical in the veterinary and human medical fields. Surgeons provide treatment to patients but can also play an essential role in advancing research through the collection of samples and recruitment of cases. One of the challenges as a surgeon is the desire to contribute to research and answer important basic science questions while still working to constantly evolve and refine surgical techniques. Due to the significant time spent on clinical training, surgeons may not receive laboratory training as part of their programs and often do not have labs, equipment or expertise to carry out this research. The important link between laboratory and clinical work is well established in medical oncology; this link to surgical oncology is less well defined but still an area of great need. Partnerships between clinicians and scientists can lead to important and significant advances in the treatment of patients, whether through the identification of new agents for intraoperative imaging or development of better local therapies for treatment, the opportunities are limitless. Collaboration between surgeons and scientist can result in significant advances to translational research and allows surgeons to provide the best and most advanced level of care to their patients, resulting in a benefit for all involved.

Afternoon Session:

6) Megan Strachan-Whaley, Byram Bridle*

Department of Pathobiology, OVC, University of Guelph

Using Viruses to Potentiate Epigenetic Modifier-Mediated Killing of Leukemic Cells

Acute lymphoblastic leukemias are caused by uncontrolled proliferation of lymphoblasts. While current treatments have increased the five year survival rate for children with B cell acute lymphocytic leukemias (B-ALL), the prognosis is worse for T cell and myeloid leukemias, and adults. Current therapies are expensive, long-term, and have a profoundly negative impact on quality of life. There are currently no treatments for feline or canine leukemias. Oncolytic viruses are effective in treating solid tumours, but perform poorly in leukemias, where the cells are widely dispersed among normal tissues with anti-viral defenses that can quench the infection. Epigenetic modifiers can modulate the replication of oncolytic viruses in solid tumours to enhance efficacy. In a reversal of this paradigm, we propose that only limited infection with an oncolytic virus will induce a robust anti-viral response that can sensitize leukemic cells to the cytotoxic effects of epigenetic modifiers. Oncolytic viruses and viral mimics in combination with epigenetic modifiers, including histone deacetylase inhibitors and azanucleosides are being tested in mouse and human leukemia cell lines. Optimal combinations will be evaluated in primary canine and feline leukemic cells and in an immunocompetent, syngeneic murine model

of B-ALL. We observed that the histone deacetylase inhibitors suberoylanilide hydroxamic acid (SAHA) and entinostat (MS-275), the azanucleosides 5-azacytidine and 5-aza-2'-deoxycytidine, oncolytic maraba, vesicular stomatitis and Newcastle disease virus all had significant efficacy as monotherapies. In addition early results suggest increased efficacy when each of the epigenetic modifiers is combined with these oncolytic viruses.
Supported by: OVC Scholarship

7) Nariman Deravi¹, Olaf Berke², Dorothee Bienzle^{1*}

Departments of ¹Pathobiology & ²Population Medicine, OVC, University of Guelph.

Prognostication of Canine T-Cell Lymphoma

Lymphoma is a common cancer in dogs frequently treated with chemotherapy. T-cell lymphoma has been thought to have poorer response than B-cell lymphoma. However, there are different types of T-cell lymphoma with purportedly variable prognosis. Lymphocytes are grouped broadly into B- or T-cell types by detection of CD79 or CD21 (B) and CD3 (T) markers using immunohistochemistry or flow cytometry. Additional markers such as CD4 and CD8 allow subclassification of T-cells. We hypothesized that specific T-cell lymphoma subtypes have distinct responses to chemotherapy and survival times. Flow cytometric data for 4 markers on samples from 111 dogs with T-cell lymphoma treated at the Ontario Veterinary College over the past 12 years were analyzed. Median overall survival time was 28.0 weeks. Twenty percent of dogs were censored (died from causes other than lymphoma or are still alive). About 20% of dogs died or were euthanized due to clinical deterioration within the first 4.5 weeks after diagnosis; 25% survived more than a year after start of therapy, and none died of lymphoma after surviving for two years. Small body size ($p=0.049$), low CD3 ($p=0.028$), absent CD4 ($p=0.002$) and high CD8 ($p=0.004$) expression were associated with longer survival than medium or large body size, high CD3, high/low CD4 or low CD8 expression respectively. Results suggest that immunophenotype correlates with survival, that small-size dogs are more likely to have longer survival, and that some types of T-cell lymphoma have a very favourable prognosis. Future investigations will relate anatomic location with immunophenotype and progression-free interval.

8) Portia Kalun, Kelly Meckling*

Department of Human Health and Nutritional Sciences, CBS, University of Guelph

Knockdown and knockout of the 1,25 D3-MARRS receptor inhibits monocytic differentiation of NB4 leukemia cells

1,25 Dihydroxyvitamin D3 (1,25 D3) primes NB4 promyelocytic leukemia cells to differentiate along the monocytic/macrophage lineage through a non-genomic mechanism. The 1,25 D3-MARRS (Membrane-Activated Rapid Response to Steroids) receptor, a recently identified membrane receptor for 1,25 D3, may be necessary for differentiation along this pathway. When NB4 cells are induced to differentiate by treatment with 1,25 D3 followed by 12-O-tetradecanoylphorbol-13-acetate (TPA), 1,25 D3-MARRS localizes to the nucleus. The goal of the current project is to establish the role of 1,25 D3-MARRS in NB4 cell differentiation along the monocytic/macrophage lineage. Using lentiviral shRNA constructs, two clones expressing shRNA against MARRS (knockdown and knockout) and a non-targeted control construct (NT)

have been established. Each cell line was treated with 100% ethanol (control), 1,25 D3 alone, TPA alone, or a combination of 1,25 D3 and TPA. Cells were assessed for markers of differentiation including non-specific esterase and phagocytic activity. While the parental (Regular) and NT cells showed an increased percentage of differentiated cells following treatment with 1,25 D3 and TPA compared to TPA alone, the knockdown and knockout lines showed decreased percentage of differentiated cells. This suggests that 1,25 D3-MARRS is required for differentiation of NB4 cells along the monocytic/macrophage lineage. Once these results are confirmed, an investigation of signalling partners of 1,25 D3-MARRS will potentially identify targets for novel promyelocytic leukemia treatments.

Supported by: NSERC

LUNCH & POSTER SESSION 11:35-1:20

OVC LLC Room 1707 B & C

Posters will be displayed all day; authors please attend your poster 12:00-1:00

POSTER ABSTRACTS (*study leader)

1) Investigating KIT and VEGF protein expression in canine prostate cancer

Carlos E. Fonseca-Alves, Priscilla E. Kobayashi, Renée Laufer-Amorim*

Department of Veterinary Clinic, University of São Paulo State - UNESP

Canine prostate cancer (PC) is an uncommon disease with highly undifferentiated morphology and has aggressive behavior associated with high metastatic rate. In veterinary medicine, there have been no new therapeutic options proven to be effective for the treatment of PC. Toceranib phosphate has activity against tyrosine kinase receptors and carcinomas expressing VEGF or have aberrant VEGF signaling are potential candidates for this therapy. Due to poor prognosis of dogs with PC and the absence of an effective primary therapy for patients, our aim was to investigate Kit and VEGF protein expression in canine PC. Thirty paraffin blocks and fourteen frozen tissue from adult intact dogs were evaluated by immunohistochemical (IHC) and Western-blotting (WB) analysis using Kit and VEGF antibodies. The IHC analysis was assessed by the distribution of positive cells for each antibody. Normal epithelial cells were negative for VEGF and 94.75% (18/19) of tumors showed VEGF positive expression. The WB confirmed the IHC results where we found higher expression of VEGF in tumors compared to normal samples ($p=0.003$). Four normal samples (40% - 4/10) showed low cytoplasmic staining of Kit and 60% (6/10) was negative. Seven tumor samples (35% - 7/20) had moderate to strong Kit staining and 65% (13/20) was negative. WB identified Kit expression in normal and PC samples. Our results indicate that neoplastic cells produce VEGF and some tumors show positive Kit expression and the use of tyrosine kinase inhibitor may be an option in the treatment of dogs with PC.

Supported by: Sao Paulo Research Foundation

2) Characterization of the TGF β -TAZ Signalling Axis in Canine Osteosarcoma Cell Lines

Adam D. Andrade¹, Pavel Neogi¹, Tony Mutsaers¹, Geoffrey Wood², Alicia Vilorio-Petit¹

Departments of ¹Biomedical Sciences and ²Pathobiology, OVC, University of Guelph

Osteosarcoma (OSA) is the most common primary bone tumor in both humans and dogs. In the later, it mostly occurs within the appendicular skeleton, and usually metastasizes to the lungs. Metastatic canine OSA is resistant to chemotherapy and is responsible for patient mortality. The factors responsible for OSA metastasis and chemoresistance are generally unknown. Transforming growth factor- β (TGF β) is a highly conserved cytokine with demonstrated roles in bone development, bone disorders, and cancer metastasis and chemoresistance. The action of TGF β is carried out through the R-Smad transcription factors, which rely on TAZ to alter gene expression. TAZ is a highly conserved transcriptional coactivator and an integral part of the Hippo pathway controlling organ size, proliferation and differentiation, as well as bone mass and osteoblastogenesis. Additionally, TAZ was shown to mediate self-renewal capacity and cancer metastasis. Based on these evidences, we hypothesized that the TGF β -TAZ signalling axis mediates OSA progression and chemoresistance. To demonstrate this, we first established the functionality of TGF β signalling in a panel of canine OSA cell lines generated by our group.

Next, we treated the cells for 2, 24 and 48 hours with 0, 0.5 and 5 ng/mL TGF β 1, and determined the pattern of Smad2 activation and TAZ expression using immunoblotting. All cell lines expressed TGF β receptors and showed a robust TGF β 1 response, displaying dose-dependent Smad activation at all time points. However, the sustainability of this response over time varied depending on the cell line. A dose-dependent TAZ increase was also observed in the majority of cell lines after 24 hours treatment with TGF β 1. Taken together, these results suggest that TGF β signalling modulates TAZ expression in canine OSA. Future studies will aim to identify the role of the TGF β -TAZ signalling axis in chemotherapy resistance.

Supported by: NSERC; OVC Scholarship

3) Assessing the use of PI3-Kinase inhibitors to rescue TGF β tumor suppressive function in advanced breast cancer

Vritika Batura, Mathepan Mahendralingam, Geordon Avery-Cooper, Mahmoud Yousseff, Alicia Vilorio-Petit*

Department of Biomedical Sciences, OVC, University of Guelph

Transforming growth factor beta (TGF β) plays a paradoxical role in breast cancer; whereby it induces apoptosis and inhibits growth in normal tissue and/or early stage tumours. However, it promotes proliferation, cell survival and invasiveness in late stage tumours. To elicit its effects, TGF β signals via canonical (Smad) and non-canonical pathways, including phosphatidylinositol-3-kinase (PI3K) and Par6. Both pathways facilitate epithelial-to-mesenchymal transition (EMT), a phenomenon implicated in metastasis. In addition to promoting EMT, our previous findings indicate that the Par6 pathway also mediates TGF β -induced apoptosis in mammary cells. Preliminary analysis of phospho-Akt levels in lysates derived from Mammary murine mammary gland (NMuMG) cells treated with a PI3K inhibitor (LY294002) suggests that high levels of Par6C, which favors Par6 signalling, promotes apoptosis via downregulation of PI3K/Akt activity. We hypothesize that in advanced breast cancers, with active TGF β signalling and high Par6 expression; PI3K inhibitors can rescue TGF β tumor suppressor function. We screened 4 advanced human breast cancer cell lines for evidence of autocrine TGF β signalling and Par6 overexpression. T47D cells, with high autocrine TGF β signalling and Par6C expression were chosen to assess the effect of PI3K inhibition on cell viability and apoptosis, using a WST-1 assay and immunoblotting, respectively. PI3K inhibition reduced cell viability and promoted apoptosis of T47D cells. Demonstrating the actual dependence of the observed effect on a functional TGF β -Par6 signalling axis using a wide range of cell lines is necessary to fully validate our hypothesis. If validated, the proposed concept might rapidly impact the treatment of breast cancer, where PI3K signalling inhibitors are already undergoing clinical trials.

Supported by: The Banting Research Foundation; NSERC

4) Colorectal cancer cells rendered resistant to the topoisomerase inhibitor SN-38 display altered interactions with fibronectin

Spencer I.T. Berg, Murray J. Cutler, Jonathan Blay*

School of Pharmacy, University of Waterloo

Elements of the local tumour microenvironment can have a profound effect on the behaviour, survival, and progression of a cancer. The extracellular matrix (ECM) is an important component of this microenvironment which can provide an initial barrier against cancer cell metastasis, or

initiate intracellular signaling cascades upon ligation with surface receptors. The purpose of this study was to investigate how colorectal cancer (CRC) cells alter their interaction with the ECM upon attaining drug resistance, and how these changes can subsequently affect cellular behaviour and resistance to chemotherapy. For this purpose, we created derivatives of the CRC cell line HT29 that are resistant to SN-38, the active metabolite of the clinically-used chemotherapy agent irinotecan. We have employed western blotting, immunofluorescence, and real-time PCR to analyze the expression of various ECM components and members of their major cellular receptors, the integrins, in a representative line of our drug resistant derivative, HT29-S, in comparison to the parental HT29 line. HT29-S exhibits a dramatic increase in adhesive capacity for fibronectin, as well as an increased ability to form fibronectin matrices in three-dimensional culture conditions. In addition, the profile of integrins expressed by HT29-S is significantly altered from the parental HT29 cells, including several known fibronectin receptors. We have also found evidence of altered intracellular signaling and ability to resist other cellular stressors in HT29-S, consistent with integrin-mediated activation of pro-survival pathways. By understanding these resistance mechanisms and how cancer cells change upon attaining drug resistance, it may be possible to design new treatment options to specifically target the drug-resistant population.

Supported by: NSERC

5) Prognostic Value of Vascular Endothelial Growth Factor Receptor 2 Expression by Neoplastic Canine Mast Cells

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Mast cell tumours (MCTs) are one of the most common skin tumours in dogs (up to 25% of skin tumours). Better prognostic and therapeutic targets are required for dermal mast cell tumors that are 'Patniak' Grade II and either 'Kiupel' low or high grade tumours, and subcutaneous mast cell tumors with a mitotic index >4 p10HPF. Vascular endothelial growth factor receptor 2 (VEGFR2), primarily expressed by vascular endothelial cells, is important for angiogenesis. However, neoplastic cells of MCTs also express this receptor and its ligand, VEGF, suggesting an autocrine role in MCT growth and survival. To investigate the prognostic value of VEGFR2 expression by neoplastic cells of MCTs, 20 subcutaneous MCTs were immunohistochemically evaluated and compared to clinical outcome. In addition to VEGFR2-staining by vascular endothelial cells, neoplastic mast cells exhibited variable positive immunoreactivity in membranous, cytoplasmic, nuclear, and perinuclear subcellular localizations. A semi-quantitative analysis was performed to determine the proportion of VEGFR2 positive pixels, VEGFR2 positive neoplastic cells, and neoplastic cells with perinuclear staining pattern. There was a strong, significant correlation between the pixel area and proportion of positive cells ($p = 0.0014$). High VEGFR2 expression by neoplastic cells was significantly associated with a decreased disease-free interval (median DFI 105 vs 931 days) and reduced overall survival (median OS 215 vs 1947 days); the proportion of neoplastic cells expressing perinuclear VEGFR2 was not associated with clinical outcome. Thus, VEGFR2 expression by neoplastic cells reflects more aggressive disease and may prove a useful prognostic and predictive marker for canine MCTs.

Supported by: OVC Pet Trust

6) Low hexokinase II expression is associated with early disease progression and worse survival in human colorectal cancer

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Enhanced glycolysis is observed during tumorigenesis, with an upregulation in key glycolytic enzymes. Hexokinase II (HKII) catalyzes the first irreversible step of glycolysis and is often overexpressed in tumors. The aims of this study were to assess the expression of HKII in colorectal cancer tissues, and to correlate HKII expression with clinicopathological characteristics and patient outcome. In total, 60 FFPE samples of different colorectal cancer tumors were obtained from the Ontario Tumor Bank, OICR. Samples were dual immunostained for HKII and carbonic anhydrase IX (CAIX; serving as an ischemic marker). Expression was assessed using semiquantitative immunofluorescence. A variation of the H-Score quantification analysis (denoted as F-Score) was developed and utilized for this study. Associations of HKII and CAIX levels with patient characteristics, tumor pathology and clinical outcome were studied using univariate analysis. HKII expression was found in neoplastic cells of non-ischemic regions of tumors and within the tumor stroma. CAIX expression was found primarily in neoplastic cells. CAIX levels were associated with patient age (OR=4.04, ≥ 70 vs. < 70). While HKII expression did not correlate with any clinical parameters, tumor samples scoring lower for HKII (F-Score < 25) were associated with early disease progression (P=0.016) and poor overall survival (P=0.025) of patients with colorectal cancer. The inverse correlation found between HKII expression and survival is counter intuitive and warrants further investigation of HKII's role in colorectal cancer progression.

Supported by: NSERC; Canadian Cancer Society Nick Natale Innovation Grant; OGS

7) Knockdown of the active glucose transporter SGLT2 in epithelial ovarian cancer (EOC) cells increases tumor size and aggression

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Tumor cells have an altered metabolism that requires consumption of a large volume of glucose for growth and survival. In vivo, we have shown that an elevated availability of blood glucose alone is sufficient to increase tumor growth and decrease overall survival. We have identified a member of the active sodium glucose transporter family (SGLT2) in mouse and human EOC cells, in addition to members of the passive transporter (GLUT) family. We hypothesize that SGLT2's unique ability to work against a concentration gradient promotes tumor aggression by providing an additional, alternative pathway for glucose uptake. We used shRNA to knock down protein expression of SGLT2 in EOC cells that had been cultured in either a normoglycemic or hyperglycemic environment. In vitro we have previously shown that cells grown in hyperglycemic environments are more metabolically active. Tumors were formed by injecting cells under the ovarian bursa of C57bl/6 mice using the orthotopic, syngeneic EOC model developed in our lab. Surprisingly, we found that tumors with SGLT2 knocked down show much more aggressive disease than tumors with normal levels of SGLT2, with mice surviving 86.4% longer. Additionally, tumors formed from cells originally cultured in normoglycemic conditions reduce mean mouse survival by 20%. Evaluation of tumor size, survival times, and incidence of

secondary tumors indicate that primary tumor size is a less accurate indicator of morbidity and mortality than is the spread of abdominal disease. The effects of reduced expression of SGLT2 suggests that SGLT2 has a protective role against EOC tumor development.
Supported by: CIHR; CIHR Vanier Canada Graduate Scholarship

8) Plant- and marine-derived n-3 polyunsaturated fatty acids prevent mammary tumor development

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Marine-derived n-3 polyunsaturated fatty acids (PUFA), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have been shown to inhibit mammary carcinogenesis. However, evidence regarding α -linolenic acid (ALA), a plant-based and major n-3 PUFA in the Western diet, remains equivocal. Previous studies have not directly compared the effects of plant and marine based n-3 PUFA on cancer outcomes. Therefore, this study examined the relative inhibitory potency of plant versus marine based n-3 PUFA on mammary tumor development in a relevant model of human breast cancer over expressing human epidermal growth factor receptor 2 (HER2/neu). Heterozygous MMTV-FVB males were bred with FVB female mice and fed one of four diets: 1) 10% safflower oil (n-6 PUFA, control diet), or 2) 10% flaxseed oil, or 7% safflower oil plus either 3) 3% flaxseed oil, or 4) 3% menhaden oil. Transgenic female offspring were maintained on the parental diet for 6 or 20 weeks. Mice fed with 10% flaxseed and 3% menhaden oil significantly decreased the number and density of terminal end buds at 6-week of age compared to the 10% safflower fed mice ($p < 0.05$). At 20 weeks of age, a significant ($p < 0.05$) dose-dependent reduction of tumour volume (24% and 78%) and multiplicity (15% and 43%) was observed in mice fed 3% or 10% flaxseed oil, respectively. Relative to 3% menhaden oil, 10% flaxseed oil resulted in a similar reduction in tumour volume (78% and 72%) but led to a greater reduction in multiplicity (27% vs. 43%). However, 3% flaxseed oil had weaker inhibitory potency compared to 3% menhaden oil ($p < 0.05$). Feeding n-3 PUFA diets significantly reduced n-6 PUFA and enriched n-3 PUFA incorporation in the plasma membrane of tumors, adjacent mammary glands, as well as serum ($p < 0.05$). Intake of 3% menhaden and 10% flaxseed oil remarkably down-regulated the expression of the proteins involved in cell proliferation (i.e. Her2, pHer2, pAkt and Ki67; $p < 0.05$) in mouse mammary tumors; and also increased the abundance of cleaved caspase-3 ($p < 0.05$), which plays an important role in cell apoptosis. However, 3% flaxseed oil was less potent in regulating these cell proliferative and apoptotic protein targets. The dose-dependent effect of flaxseed oil clearly demonstrates a role for ALA in cancer prevention. However, it is not possible to determine whether this effect is directly due to ALA or the conversion to EPA and DHA. Overall, this study demonstrates that while marine-based n-3 PUFA are more potent than plant-derived ALA, lifelong exposure to n-3 PUFA, whether from marine or plant source, can mitigate tumor outcomes.

Supported by: CIHR

9) Targeting the Wnt pathway in zebrafish as a bioassay to identify novel therapeutic compounds.

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Deregulation of Wnt signaling pathway has been implicated in numerous diseases. It is well known that greater than 90% of sporadic colon cancers are initiated by APC or β -catenin mutations, both leading to Wnt pathway activation. Therefore, identifying small molecule inhibitors of this pathway is of critical importance towards identifying clinically relevant drugs. Here, we used an in vivo phenotype-based chemical suppressor screen to identify potential Wnt inhibitors. Basically, treatment of zebrafish embryos with the chemical BIO results in hyperactivation of the Wnt signaling pathway and an eyeless phenotype at 1 dpf. We screened several small molecule libraries to identify molecules that would rescue (suppress) the eyeless phenotype. When eye development was restored, the test compound was classified as a positive candidate as a Wnt pathway inhibitor and a suppressor index was calculated based on the percentage of the eyes rescued by the test compound and BIO. Here we describe our results from our two most recent screens. The first library is composed of 37 compounds derived from anthranilic acid and acridones, compounds with various pharmaceutical activities (MA1-MA37). The second library consists of 357 food-derived compounds (UW1-UW357). Using this approach we identified 4 molecules with a high suppressive index (>50) (ie., rescued the BIO-induced eyeless phenotype). A further 8 compounds had a moderate suppressive index (30-50). None of these molecules showed toxic effects. Therefore, combining the efficacy, sensitivity, and stringency of our in vivo bioassay, we can identify potentially clinical relevant drugs in a rapid and cost-effective way.

Supported by: CAPES

10) Evaluation of the TGF β -TAZ signaling axis in canine osteosarcoma tumours: optimization of immunohistochemistry protocols

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Canine osteosarcoma (OS) is a commonly diagnosed bone tumour, with a majority of cases observed within the appendicular skeleton in large-breed dogs. OS prognosis is generally poor, due to its highly metastatic nature. Identifying new prognostic and potentially targetable mediators of OS is necessary to improve clinical outcome. Transforming growth factor beta (TGF β) mediates skeletal development and cancer metastasis. We previously demonstrated that TGF β induces the expression of TAZ, a transcription factor involved in bone development, the control of cell size and stem cell renewal. TAZ has been previously associated with metastasis and poor prognosis, and we observed that TAZ mediates chemoresistance in OS cell lines. Based on this, we hypothesized that TAZ levels will correlate with survival and metastatic burden in OS patients. To begin addressing this hypothesis, we optimized immunohistochemical protocols for the detection of TAZ and phosphorylated Smad2 (pSmad2), an indicator of active TGF β signaling. Sodium Citrate (pH = 6.0) antigen retrieval at 95°C for 10 and 15 minutes, and

antibody concentrations of 1:200 and 1:800, were optimal for TAZ and pSmad2, respectively. Using these conditions, we immunolabeled a tissue microarray (TMA) comprised of 88 cores from a variety of canine tumour samples, as well as 13 cases of normal canine tissue. pSmad2 immunolabeling was nuclear, as expected. However, pSmad2 positivity was highly variable among different OS specimens. TAZ localized to both the nucleus and cytoplasm, and there was a general trend toward positive, albeit variable labeling among all OS tumors assessed. Future studies will involve scoring of pSmad2, TAZ and downstream mediators of stemness to investigate the prognostic and/or predictive value of the TGF β -TAZ signaling axis and/or individual components of this axis.

Supported by: OVC Pet Trust

11) In vitro effects of epidermal growth factor receptor kinase inhibition on radiation response in canine osteosarcoma cell lines

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Introduction: External beam radiation therapy plays an important role in the palliative treatment of canine osteosarcoma, with transient improvement in analgesia observed in the majority of cases. The addition of radiation sensitizing agents may further improve outcomes for these patients. Epidermal growth factor receptor (EGFR) expression has been documented in canine osteosarcoma and correlated to prognosis. However, effects of EGFR inhibition in radiation responsiveness have not been characterized. This study examined the effects of the small molecule EGFR inhibitor erlotinib on canine osteosarcoma radiation responses, target and downstream protein expression in vitro. Methods: Canine osteosarcoma cell lines D-17, Abrams, and Dharma were utilized. Radiation was delivered by a Varian trilogy linear accelerator. Doses of 0,2,4,6,8,10 Gy were utilized for clonogenic survival assays, and colonies were stained with crystal violet and enumerated after 10-14 days. Cell lysates were also obtained following 2 Gy radiation at time points from 0.25 to 72 hours for protein profiling. Cell viability, clonogenic survival and phospho/total EGFR, HER2, Akt, and VEGF production assays were conducted to assess single agent and combination treatment efficacy. Results: Erlotinib in low micromolar doses inhibited clonogenic survival as a single agent treatment, and further inhibited clonogenic survival in cells following radiation. Radiation stimulated phospho-Akt, which was inhibited by the inclusion of erlotinib. VEGF in conditioned media was increased following both radiation and especially erlotinib treatment. Conclusion: Preliminary results in canine osteosarcoma cell lines suggest there may be a role for EGFR inhibition to improve canine osteosarcoma treatment responses to radiation therapy.

Supported by: Department of Clinical Studies

12) Analysis of Munc18c and Syntaxin4 Function During Tumor Cell Invasion

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Dissolution of the extracellular matrix (ECM) is a prerequisite for tumor cell invasion and the establishment of metastases. The expression of matrix metalloproteinases, including membrane type 1-matrix metalloproteinase (MT1-MMP) has been linked to the capacity of invasive carcinoma cells to invade surrounding tissue. MT1-MMP has been shown to localize to degradative structures, termed invadopodia, acting to initiate cell invasion. Membrane trafficking of invadopodium-associated proteins is hypothesized to regulate their formation and invasive behavior. A protein family that has a major role in vesicle trafficking, SNAREs (soluble N-ethylmaleimide-sensitive factor activating protein receptors), function to localize vesicles to target membranes. Recent evidence has shown that the SNARE complex responsible for the transport of MT1-MMP is comprised of Syntaxin4, SNAP23, and VAMP7 in invasive MDA-MB-231 cells. Additionally, the formation of this complex correlated with a decrease in Syntaxin4 serine/threonine phosphorylation. To elucidate the importance of this dephosphorylation, a well-known SNARE interacting partner, Munc18c, has been functionally assessed in the context of invadopodium-mediated ECM degradation. Immunocytochemistry of cells plated onto an ECM substrate indicates that Munc18c localizes to the plasma membrane and around the nucleus. Furthermore, Munc18c localizes to the adhesion ring around maturing invadopodia. Co-immunoprecipitation of Munc18c from cells forming invadopodia shows an interaction with phosphorylated Syntaxin4, but this interaction does not change relative to cells faced with a non-ECM substrate. Additionally, RNAi-mediated knockdown of Munc18c impairs invadopodium formation and by extension ECM degradation. Data suggests Munc18c preferentially interacts with phosphorylated Syntaxin4, preventing irregular SNARE complex formation.

Supported by: NSERC

13) Canine splenic hemangiosarcoma treated with splenectomy and 6 doses of dose-intensified doxorubicin chemotherapy with or without metronomic cyclophosphamide

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This retrospective study investigated the outcome of 36 dogs with splenic hemangiosarcoma (HSA) treated with splenectomy and dose intensified doxorubicin followed with or without metronomic (LDM) cyclophosphamide maintenance therapy. Clinical stage was available for 33 dogs (5 Stage I, 18 stage II and 10 stage III). Nine dogs had macroscopic and 24 dogs had microscopic disease at the start of doxorubicin. Nineteen dogs received LDM cyclophosphamide. Thirty-three dogs died of HSA, 1 of dilated cardiomyopathy (day 148), 1 was alive (day 924) and 1 lost to follow-up (day 398). Median overall survival (OS) and progression free survival (PFS) were 131 and 119 days. Clinical stage and tumor burden (microscopic vs. macroscopic) at the start of chemotherapy was prognostic for PFS. No significant difference was observed for addition of LDM cyclophosphamide after a completed doxorubicin protocol. A randomized prospective trial would be necessary to evaluate the impact of additional LDM therapy.

Supported by: Department of Clinical Studies

14) Par6 phosphorylation at Ser345 mediates invasion and metastasis of HER2-transformed mammary cells

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The HER2 oncogene is overexpressed in a subset of breast cancers, and was previously shown to cooperate with TGF β in promoting breast cancer invasiveness, migration and metastasis. TGF β is a well-documented inducer of epithelial-mesenchymal transition (EMT), a process of cellular plasticity implicated in metastasis, whereby epithelial cells lose their typical phenotype, including apical-basal polarity, and acquire fibroblastic features, such as a flexible cytoskeleton, motility, invasiveness and stemness. TGF β induced-EMT requires canonical (Smad) and non-canonical signalling, the later including, but not limited to, activation of the Par6 pathway. Par6 is an essential component of a complex that localizes to the tight junction and participates in the establishment of apical-basal polarity. Overexpressed HER2 was previously shown to interact with Par6 to promote cellular survival in breast cancer cells, and TGF β was previously observed to induce Par6 phosphorylation at Ser345, to ultimately promote the dissolution of tight junctions and rearrangements of the actin cytoskeleton, both of which are essential for EMT and TGF β -mediated breast cancer metastasis. Here we addressed the hypothesis that Par6 phosphorylation at Ser345 is important for the EMT-promoting cooperation between HER2 and TGF β . For this purpose, we employed standard and collagen I-matrigel three-dimensional culture models of mutant HER2- transformed NMuMG murine mammary cells (NMu/HER2) expressing Par6 wild type or a dominant negative S345A Par6 mutant, and orthotopic allografts of the aforementioned cells in CD17/SCID mice. Protein expression and signaling activation was analyzed by immunoblotting and immunofluorescence in cultured cells, and by immunohistochemistry in tumor tissue. Non-parametric and parametric ANOVA were used to assess statistical significance. We observed that Par6 phosphorylation at Ser345 promotes in vitro invasiveness of HER2-transformed cells both independently and in response to TGF β . Blockade of Par6 phosphorylation at Ser345 significantly reduced the number of metastatic colonies/lung and overexpression of Par6 wild type significantly increases the number of metastases per lung. Immunoblotting and Immunohistochemical analysis of cultured cells and tumor tissue, respectively, showed that Smad2 activation status was equivalent in Parental, Par6 wild-type and Par6 S345A expressing cells and tumors, suggesting that Par6 signalling mediates the pro-metastatic cooperation of HER2 and TGF β in a Smad-independent manner. This is the first study to implicate Par6 in HER2-driven invasion and metastasis, and supports the notion that Par6 signaling targeting might be considered to control dissemination of HER2-dependent cancers. Supported by: OVC Bull Fellowship; NSERC

15) Investigation into the Regulation of Wnt Signaling by Axin2 during Development of Zebrafish

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The canonical Wnt signaling pathway is an evolutionarily conserved pathway crucial in the regulation of key aspects of embryonic development such as cell migration, proliferation, differentiation and embryonic patterning. It is also involved in adult stem cell homeostasis and abnormal regulation of this pathway leads to developmental defects and disease, including

cancer. Two universal targets of Wnt signalling are the negative feedback inhibitors Nkd1 and Axin2 that we speculate control the intensity and duration of Wnt signalling, respectively. In both cases, we hypothesize that they function as a rheostat, preventing major fluctuations in the Wnt signal. However, in some cancers these feedback inhibitors are present in cells with increased Wnt activation but appear to be non-functional. We use zebrafish as a developmental model to investigate the effects of Axin2 loss of function. We predict that under normal developmental condition loss of Axin2 should have little or no effect on zebrafish embryos; however, overexpression of Wnt ligand in zebrafish lacking Axin2 should severely alter the embryos development. We used CRISPR/Cas9 genome editing to produce zebrafish lacking Axin2 and demonstrated that Axin2 knockout zebrafish are hypersensitive to Wnt signaling. Therefore, our research model may provide a useful tool with which to study Wnt related diseases and pathway regulation in normal developmental processes, particularly how loss of Axin2 function contributes to disease.
Supported by: NSERC

16) Owner attitudes and practices regarding the nutrition of their dog diagnosed with cancer
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Objective - To investigate owner attitudes and their respective dietary practices following the diagnosis of cancer in their dog. **Design** - Cross-sectional survey. **Sample** - Seventy-five dog owners and their dogs presenting with a cancer diagnosis to a tertiary referral oncology service. **Procedures** - Completion of a written demographic survey along with one-on-one in-person or telephone interviews conducted using a standard questionnaire with open- and closed-ended questions regarding the canine cancer patient's nutrition. **Results** - Conventional diets (71%) were the most commonly fed as a single diet to canine cancer patients followed by homemade cooked (7%), and homemade raw (4%) diets. Several owners (18%) were providing combinations of the aforementioned diets. Owners tended to exhibit some distrust towards conventional diets following the cancer diagnosis. Appetite loss, which occurred in 35% of dogs, was found to be predictive of a diet change (Odds Ratio=3.42, P<0.05). Diet changes reported for 25% of dogs involved exclusion of conventional (58%) and/or inclusion of homemade (43%) components. Ninety percent of diet changes were associated with the cancer diagnosis. Supplements were given by 39% of owners. Eighty-five percent of owners highly valued veterinarians as a source of nutritional advice. **Conclusions and Clinical Relevance** - Following the diagnosis of cancer, a number of dog owners appear to change their approach to managing their dog's nutrition. Given the high value dog owners place on a veterinarian's nutritional advice, veterinarians have a key role in assisting owners in managing their dog's nutrition following a diagnosis of cancer.

17) Knockdown of Autophagy/ Mitophagy Genes Sensitizes Leukemia Cells to Avocatin B-Induced Cell Death

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Recent studies have shown that leukemia cells possess mitochondrial alterations characterized by increased mitochondrial mass and a reliance on fatty acids for survival. We recently

demonstrated that the novel agent avocatin B preferentially induced death in leukemia cells and leukemia stem cells by exploiting these mitochondrial characteristics whereby avocatin B accumulated in mitochondria and inhibited fatty acid oxidation resulting in ROS-mediated apoptosis. Here, we sought to better understand the link between genes involved in mitochondrial maintenance and avocatin B-induced cell death in leukemia cells. Knockdown of autophagy/mitophagy genes NIX (gene: BNIP3L) and p62 (gene: SQSTM1) produced cells that were more sensitive to avocatin B-induced death. Mechanistically, this increased sensitivity was linked to increased levels of endogenous ROS levels caused by the genetic knockdown. Understanding the role of autophagy/ mitophagy in leukemia will provide the framework necessary for improved drug design and may provide a future prognostic tool to identify patients who may benefit from mitochondria target drugs.
Supported by: Leukemia and Lymphoma Society of Canada; Canadian Hematology Society

18) Estrogen receptor is a target for novel leukemia stem cell therapeutics

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Acute myeloid leukemia (AML) is a devastating disease with only 5-35% of adult patients surviving past 2 years. Leukemia stem cells (LSCs) are a promising target for future AML therapy, as they are responsible for disease onset, propagation and patient relapse. To identify potential novel anti-LSC therapeutics, we created and screened a unique library consisting of 288 food-derived bioactive compounds with previously unrecognized anti-cancer activity. Here, we identified diosmetin (dios), a flavonoid found in various plants, as a novel anti-LSC agent. Dios significantly reduced the viability of TEX AML cells, which possesses LSC properties (EC50: 7 μ M), with no reduction in viability in our non-LSC control cell line. Dios induced cell death through apoptosis as a result of the activation of caspases3/7/8. The chemical structure of dios indicates it as possible as a phytoestrogen and as such we investigated the estrogen receptor (ER) as the potential molecular target for dios induced leukemia cell death. Coincidentally, cell lines sensitive to dios displayed an increased expression of ER- β protein compared to insensitive cell lines. However, this pattern was not observed for the expression of ER- α . ER agonist and antagonist reporter assays demonstrated that at lower dios concentrations (1.58-12.5 μ M), acts as an agonist in ER- β reporter cells. In summary, these studies highlight a novel AML therapeutic strategy through the activation of ER- β by dios resulting in leukemia cell apoptosis.
Supported by: University of Waterloo; Canada Stem Cell Network

19) Clinical Research in Companion Animals with Cancer

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Although cancer survival rates have been steadily increasing over the past decade, cancer is still responsible for ~76,000 cancer deaths in Canadians annually. A critical component in the development of new therapies is the use of model systems. Tumour cell lines and genetically engineered and/or immunodeficient mice, whilst valuable research tools, possess shortcomings

that may partly explain the poor success rate in translating basic findings to improved clinical outcomes. In contrast, cancers in companion animals offer several advantages over conventional models: spontaneously arise, shared environment, genetically and histologically heterogeneous, develop in the context of an intact immune system, receive comparable treatments, and clinical progression often mirrors the human disease. Thus companion animal cancer patients offer significant potential to fill the critical gap that exists between preclinical and phase I/II studies whilst providing benefits to animal patients in terms of cutting-edge techniques and new treatment options. The Mona Campbell Centre for Animal Cancer (ACC) receives referrals of ~1440 patients/year (1240 dogs; 200 cats) and draws from ~1500 veterinary practices located throughout southern Ontario and beyond. The most common cancer types referred are lymphoma, mast-cell tumour, osteosarcoma, soft tissue sarcoma, mammary cancer, hemangiosarcoma, thyroid cancer and melanoma. Currently, there are 12 oncology-related clinical trials recruiting patients: 8 canine, 3 feline and 1 both cats & dogs (further information: <http://ovc.uoguelph.ca/icci/trials>). Hence, ACC clinical cancer studies have the potential to improve not only the lives of veterinary patients but also to provide robust preclinical data for translational research relevant to human cancer.

Supported by: Smiling Blue Skies Cancer Fund; OVC Pet Trust

20) The ICCI Companion Animal Tumour Sample Bank: facilitating translational cancer research

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The Companion Animal Tumour Sample Bank (CATSB) aims to promote basic and translational research in the field of veterinary oncology. Located in the OVC Mona Campbell Centre for Animal Cancer, the CATSB is the only veterinary oncology tissue bank in Canada. Human biobanks are crucial in enabling translational research to improve diagnosis, treatment, and outcome for cancer patients. Modeling human biobanks, the CATSB strives to facilitate research to improve the lives of companion animals with cancer. Sample types collected and stored at ultracold temperature are: serum, plasma, buffy coat, urine, and tissue samples. Tissue samples, both tumour and matched normal, are collected immediately following surgical excision and available as flash frozen, in RNAlater, and in CryoMatrix. Tumour tissue is also formalin fixed and paraffin embedded for quality control, and assessed by pathologists to confirm diagnosis and quantify proportion of tumour captured in the sample. Currently, there are approximately 450 cases banked. The three most abundant canine tumour types and their respective amounts represented in the CATSB are: 51 soft tissue sarcomas, 49 osteosarcomas, and 45 mast cell tumours. Researchers can access samples by filling out a short application form. A cost-recovery fee is assessed to enable the CATSB to continue its mission; this fee is subsidized for University of Guelph researchers. In addition to samples, researchers receive: patient signalment, detailed histopathology reports, and clinical follow-up information is also available. Overall, CATSB allows detailed molecular analysis of companion animal cancers and links these findings to clinically relevant neoplastic behaviour to facilitate both animal and human translational cancer research.

Supported by: Smiling Blue Skies Cancer Fund; OVC Pet Trust

21) The Effects of 3TSR Fusion Proteins on Epithelial Ovarian Cancer

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Epithelial ovarian cancer is the most common and most deadly of the gynecological disorders, with late stage detection and inadequate treatment options contributing to its high mortality rate. Angiogenesis is a key mediator of cancer development, and as such represents an attractive therapeutic target for the inhibition of tumour growth. Thrombospondin-1 has been shown to have anti-angiogenic and apoptotic effects on vasculature and ovarian tumour cells. Current issues with thrombospondin-1 mimetic peptides include the short half-life of these compounds, which limits their efficacy. We therefore sought to evaluate the efficacy of a newly developed thrombospondin-1 mimetic fusion protein known as Fc-3TSR. Fc-3TSR is comprised of a linker protein that joins two peptides containing the 3 type I repeats (3TSR) of the TSP-1 gene. Initiating its anti-angiogenic and apoptotic effects through the CD36 receptor on both tumour and endothelial cells, the longer half-life of Fc-3TSR appears to significantly improve its anti-tumour properties. Results to date demonstrate an increased ability of Fc-3TSR to induce apoptosis and inhibit proliferation in ovarian tumour cells when compared to 3TSR. In addition, Fc-3TSR has been demonstrated to reduce the invasiveness of ovarian tumour cells and regulates factors important for angiogenesis and tumor cell survival. Most recently we have shown that Fc-3TSR can potentially induce regression of advanced stage ovarian cancer in a mouse model of disease, and as such we believe this compound may hold therapeutic promise for women with advanced stage ovarian cancer.

Supported by: CIHR

22) Bolus high-dose sunitinib overcomes potential for rebound metastasis following pre-surgical neoadjuvant treatment breaks

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Preclinical and clinical studies have suggested that stopping antiangiogenic therapy may lead to rapid revascularization and rebound tumor growth in certain instances. However, the implications for these findings remain unclear. It is possible that treatment rebounds may impact disease progression following neoadjuvant therapy, where drugs are administered for short periods and then halted prior to surgical removal of a primary tumor. Indeed, recent retrospective clinical studies have suggested that neoadjuvant antiangiogenic treatment discontinuation increases vascular proliferation and, in turn, might increase post-surgical disease progression and metastasis. However, this hypothesis has not been studied experimentally in clinically relevant mouse models of metastatic disease. We examined the effect of treatment discontinuation in the neoadjuvant setting using approved vascular endothelial growth factor receptor (VEGFR) tyrosine kinase inhibitors (TKIs) such as sunitinib. Our studies utilized models of spontaneous metastatic breast, melanoma, and kidney cancer following surgical removal of orthotopically grown primary tumors. Neoadjuvant sunitinib treatment periods were 14 days in duration (60 mg/kg) with treatment breaks of 0, 3, 7, and 11 days compared. Pre-surgical molecular and cellular biomarkers were investigated as potential surrogates for post-surgical metastatic disease

progression. These included tumor and vascular cell Ki67 and CD31 expression, as well as circulating tumor cells (CTCs) and myeloid derived suppressor cells (MDSCs). Our results show that VEGF RTKI neoadjuvant treatment benefits observed pre-surgically (such as reduction in primary tumor growth) did not consistently correlate with post-surgical survival and reduced metastatic recurrence. The addition of treatment breaks prior to surgery had positive or negative impact on post-surgical survival depending on the tumor model used. Interestingly, high-dose bolus (3 day) neoadjuvant sunitinib (120 mg/kg) treatment consistently improved post-surgical benefits when compared to sustained treatments, despite gaps in therapy. Finally, our results show that tumoral CD31+/Ki67+ cells, CTCs, and MDSCs may serve as surrogate markers of metastatic potential and predict the impact of treatment cessation on disease recurrence. Supported by: Roswell Park Alliance Foundation; USA Department of Defense

23) Hypoxic cancer cells exploit a specialized protein synthesis to enhance their proliferation, migration, and invasion

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Despite the diversity found in the genetic makeup of cancer cells, several cancers share the same physiological attributes known as the tumor microenvironment. Hypoxia, an aspect of the tumor microenvironment, results in the suppression of the standard protein synthesis machinery to limit energy expenditure. Hypoxic cells switch from utilizing the eukaryotic initiation factor 4E (eIF4E) to the eIF4E2 to initiate the selective translation of hypoxia-response mRNAs. Cancer cells exploit this pathway for their growth, proliferation, and survival. Here, we show that eIF4E2 drives the synthesis of various cell surface receptor tyrosine kinases (EGFR and PDGFRA) and adhesion molecules (CDH22) utilized by a panel of cancer cells to autonomously proliferate, migrate, and invade. Silencing eIF4E2 resulted in either a decrease in cell migration and invasion, or a complete inhibition of these processes. Our future goals are to link the eIF4E2-dependent synthesis of CDH22 in hypoxic cancer cells, and the hypoxic repression of CDH1 (E-cadherin), with their ability to migrate away from the primary tumour during metastasis. Our findings will answer questions regarding the biology of cancer and expand the current knowledge of genes exploited by cancer cells for metastasis and invasion. We will also highlight eIF4E2 as a potential drug target for future cancer treatments and therapies. Supported by: Canadian Cancer Society Research Institute; The Cancer Research Society

24) Characterizing Wnt signaling activity and regulation in intestinal epithelial cells

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Wnt signaling is an evolutionarily conserved pathway involved in many aspects of early development and stem cell regulation. In the intestinal crypt, Wnt signaling maintains the proliferation of the crypt stem cells to replenish the intestinal epithelium in adult organisms. Activating mutations in Wnt signaling occur within these crypt cells and are found in the majority of colorectal cancers (CRC). The Van Raay lab has previously demonstrated that in addition to activating the pathway, Wnt ligands are subsequently required for pathway

regulation. Importantly, common mutations lead to Wnt activation and oncogenesis independent of the Wnt ligand, thus preventing this control. Here we begin to explore the role of ligand dependent and independent Wnt signaling in cancer cell development, utilizing Intestinal Epithelial Cells (IEC-18s) as a progenitor cell model. Our results indicate these cells are responsive to different Wnt signaling agonists at a biochemical level and we are currently determining the physiological significance of this activation. In addition, we are also characterizing the function of Wnt ligand dependent negative feedback regulators in these epithelial progenitor cells.

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25) Examining the role of the 1,25D3-MARRS receptor in mammary gland development

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Vitamin D in its active form (1,25D3) is important for blood calcium and phosphate homeostasis, but has secondary outcomes in many tissues including cell growth inhibition and anti-cancer effects. Until recently, it was thought that 1,25D3 and its effects on gene expression were initiated solely through binding the classic vitamin D receptor. But recent findings have suggested that there is another receptor through which 1,25D3 elicits rapid responses, called the membrane-associated rapid response steroid-binding (MARRS) receptor. The purpose of this study was to examine the role of 1,25D3-MARRS in mammary gland development in a knockout mouse model. 1,25D3-MARRS was reduced in epithelial cells of mammary glands using the Cre/loxp system. 4th and 5th mammary glands were collected from 4- and 6-week old female MMTV-Cre mice (n=36). Tissue growth was assessed by counting the number of terminal end buds of alveolar branches, measuring the length of the longest branch, and measuring total mammary gland area covered by branching. Reduced 1,25D3-MARRS expression in the tissue was successfully achieved and confirmed by western blot. 4-week knockout mice had significantly less terminal end buds than knockdowns or controls of the same age (p=0.03). Although not significant, the total area covered by branching was also less in the knockouts. These results suggest that a decrease in 1,25D3-MARRS expression may cause mammary gland growth inhibition during the early stages of puberty. One possible explanation for this may be increased activity via the classic receptor in the absence of 1,25D3-MARRS, though further research is required.

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26) DNA Ministrings: A new approach to cancer gene therapy

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Gene therapy depends on the successful transfer of genetic material into the nuclei of targeted human cells. Non-viral gene therapy strategies address the safety and limited cargo capacity concerns of viral gene delivery, but generally suffer from low transfection efficiencies. We previously constructed an enhanced linear covalently closed (LCC) minivector, a DNA ministring, which confers improved efficiency and safety over its plasmid, and even its isogenic

minicircular counterparts. DNA minivectors are comprised solely of the eukaryotic expression cassette without the bulk of an immunogenic bacterial backbone, thus ensuring greater bioavailability, higher transfection efficiency, and prolonged duration of gene expression. The linear nature of DNA ministrings also minimizes the potential for insertional mutagenesis from random genomic integration. We report the construction of a novel *E. coli* platform utilizing filamentous bacteriophage (phage) M13 to produce phage-encapsulated DNA ministrings as a one-step therapeutic production platform. Phages have been previously exploited for drug delivery because they are safe, easy to produce, and genetically versatile. The filamentous phage is unique in its cylindrical and helical structure, allowing for DNA cargo of practically any length. Since M13 packages single-stranded DNA (ssDNA), the parent plasmid must produce an ssDNA molecule that encodes both the ministring base sequence as well as its complementary sequence, which then anneal to form the double-stranded DNA (dsDNA) ministring molecule prior to packaging. We report our initial advancements in developing this parent plasmid.