



9th Annual ICCI

**Cancer Research
Symposium**

Tuesday May 17, 2016
OVC LLC 9:00-5:00

Introductory Remarks

Welcome to the 9th 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. In addition to campus investigators we welcome local, national and international researchers. This year in particular we have several presentations about sarcomas, a relatively rare group of cancers in humans that are prevalent in companion animals.

Through interactions facilitated by this meeting, it is hoped that new insights and cooperation 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. Jaime Modiano, 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 partnerships for all our attendees.

Co-Organizers

Tony Mutsaers and Brenda Coomber

Clinical Studies and Biomedical Sciences, University of Guelph



Thanks to Barb Gaudette, 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: Canadian Cancer Society; Cancer Research Society; Leukemia & Lymphoma Society of Canada; Canadian Hematology Society; Canadian Breast Cancer Foundation; Terry Fox Research Institute; Canada Stem Cell Network; Public Health Agency of Canada; AAVN-Waltham Research Grant; Royal Canin; BioCanRx; Rna Diagnostics Inc; CIHR; NSERC; CFI; Canada Research Chairs; OGS; University of Waterloo; OVC Pet Trust Fund; Smiling Blue Skies Cancer Fund; Art Rouse Cancer Biology Graduate Stipend; Office of the Dean, Ontario Veterinary College.

ICCI 9th Annual Cancer Research Symposium , Tuesday May 17, 2016
Morning Session: Room 1714, OVC LLC

9:00 - 9:05 Welcome and Introductory Remarks

9:05 - 9:40 Guest Speaker

Dr. Rebecca Gladdy, Assistant Professor of Surgery, Mount Sinai Hospital, Institute of Medical Science, University of Toronto

Advancing Model Development in Soft Tissue Sarcoma: Translating Mechanisms into Therapeutic Potential

Short talks from submitted abstracts:

9:40 - 9:55

Daniel Pyc, Department of Human Health & Nutritional Sciences, CBS, University of Guelph

Effect of exercise and n-3 polyunsaturated fatty acids on mammary tumour development in mouse model of human breast cancer

9:55 - 10:15 Coffee Break and Poster Viewing Room 1707 B & C, OVC LLC

10:15 - 10:30

Megan Brasher, Department of Molecular & Cellular Biology, CBS, University of Guelph

Analysis of Munc18c and Syntaxin4 Function During Tumor Cell Invasion in MDA-MB-231 Cells

10:30 - 10:45

Nicole Kelly, Department of Molecular & Cellular Biology, CBS, University of Guelph

Hypoxia represses E-cadherin translation and activates cadherin 22 synthesis via eIF4E2 to drive the migration, invasion and adhesion of breast cancer cells.

10:45 - 11:00

Hayley Lau, Department of Molecular and Cellular Biology, CBS, University of Guelph

Investigating the molecular signaling mechanisms of the neuronal adaptor protein, ShcD, in development and cancer

11:00 - 11:10 Remarks from Jeff Wichtel, Dean of OVC

Short talks from submitted abstracts:

11:10 - 11:25

Spencer Berg, School of Pharmacy, University of Waterloo

Colorectal cancer cells resistant to the chemotherapeutic agent SN-38 gain integrin $\alpha_5\beta_1$ expression and signalling

11:25 - 11:40

Anita Luu, Department of Biomedical Sciences, OVC, University of Guelph

Prognostic value of activated Smad2 and TAZ levels in appendicular canine osteosarcoma: a pilot immunohistochemistry study in a tissue microarray

11:40 - 11:55

Jacob van Vloten, Department of Pathobiology, OVC, University of Guelph; ²St. Joan of Arc Catholic

Fever-Grade Temperatures Attenuate Rhabdovirus-Mediated Oncolysis of Cancer Cells

12:00- 1:25 Poster Session and Lunch

Room 1707 B & C, OVC LLC

Afternoon Session: Room 1714, OVC LLC

1:25 - 2:00 Guest Speaker

Dr. Urban Emmenegger, Assistant Professor, Department of Medicine, Scientist, Odette Cancer Centre, Sunnybrook Health Sciences Centre, University of Toronto

Stereotactic ablative radiotherapy (SABR) of oligometastatic prostate cancer: true clinical value or doing the feasible?

2:00 - 2:15

Vicky Sabine, Department of Clinical Studies, OVC, University of Guelph

ICCI comparative oncology program: Utilizing spontaneous companion animal cancers as models for human cancers

2:15 - 2:30

Nicole Weidner, Department of Biomedical Sciences, OVC, University of Guelph

Vitamin D status and acute phase protein concentrations in canine cancer patients

2:30 - 2:45

Shannon Wainberg, Department of Clinical Studies, OVC, University of Guelph

Comparison of a ventral cervical versus two lateral incisions for extirpation of the mandibular and medial retropharyngeal lymph nodes

2:45 - 3:00

Laura Pritzker, Rna Diagnostics, Toronto

RNA Disruption as an indicator of CHOP therapy efficacy in canine lymphoma

3:00 - 3:15

Paul Woods, Department of Clinical Studies, OVC, University of Guelph

Novel oncolytic Maraba virus for the adjuvant treatment of feline mammary carcinoma

3:15 - 3:40 Snack Break and last chance to view posters Room 1707 B & C, OVC LLC

3:40 - 4:55 Keynote Speaker

Dr. Jaime Modiano, Perlman Professor of Oncology & Comparative Medicine, Director, Animal Cancer Care & Research Program, College of Veterinary Medicine and Masonic Cancer Center, University of Minnesota, Minneapolis, MN, USA

Swimming against the current: a turning point in the battle against hemangiosarcoma

4:55 - 5:00 Closing Remarks

KEYNOTE PRESENTATION

3:40 OVC LLC Room 1714

Dr. Jaime Modiano, VMD, PhD

Perlman Professor of Oncology and Comparative Medicine
Director, Animal Cancer Care and Research Program,
College of Veterinary Medicine and Masonic Cancer Center, University of Minnesota

Swimming against the current: a turning point in the battle against hemangiosarcoma

Hemangiosarcoma (HSA) is a common, lethal tumor of dogs that has a unique breed predilection. This disease can arise in almost any anatomical site, but it is diagnosed most often in the spleen, liver, heart, and skin. While some cutaneous HSAs are treatable, almost all visceral HSAs are incurable. The ontogeny of HSA was originally assigned to cells from the endothelial lineage based on inference from its morphologic appearance. However, accumulating data indicate that HSAs originate from pluripotent bone marrow progenitors. We have developed a molecular subclassification of HSA to provide a foundation for understanding both its intrinsic character and that of the cells that comprise its microenvironment. This has also guided discovery and development of an EGF-bispecific angiotoxin (eBAT), is a novel biological consisting of a dually targeted, recombinant bacterial toxin. eBAT is safe, and it improves outcomes for dogs with HSA in the adjuvant setting. This has prompted its combination with identification of HSA cells in the blood as a novel approach for early detection and chemoprophylaxis. The presentation will begin with a brief historical perspective and review of the pathobiology of canine HSA, it will continue with an update on recent advances in the molecular and cellular features of these tumors, and it will culminate with an overview of the SRCBST clinical trials, the Shine-On study for HSA prevention, and the application of these findings to ongoing work focused on prevention and treatment of human sarcomas.

Dr. Jaime Modiano completed his veterinary training and PhD in Immunology through the Veterinary Medical Scientist Training Program at the University of Pennsylvania, followed by a residency in Veterinary Clinical Pathology at Colorado State University and a post-doctoral fellowship at the National Jewish Center for Immunology and Respiratory Medicine. He served on the faculty of Texas A&M University and the University of Colorado Health Sciences Center before joining the University of Minnesota. Dr. Modiano holds the Alvin and June Perlman Endowed Chair of Animal Oncology and is the Director of the Animal Cancer Care and Research Program of the College of Veterinary Medicine and the Masonic Cancer Center at the University of Minnesota. The research emphasis in Dr. Modiano's laboratory is to understand how and why cancer happens and to translate basic research into clinical applications that improve the health and wellbeing of companion animals and humans.

Past ICCI Symposium Arthur Willis Distinguished Speakers

2015	Nicola Mason	2011	Cheryl London
2014	Deborah Knapp	2010	Matthew Breen
2013	David Argyle	2009	Barbara Kitchell
2012	Timothy Fan		

GUEST SPEAKER

9:05-9:40 OVC LLC Room 1714

Dr. Rebecca A. Gladdy

Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Department of Surgery &
Institute of Medical Science, University of Toronto

Advancing Model Development in Soft Tissue Sarcoma: Translating Mechanisms into Therapeutic Potential

Rhabdomyosarcoma (RMS) is a common pediatric cancer that is usually treated with chemotherapy, radiation and/or surgery, however 5-year survival rates are only 30% in high-risk groups and have not improved in the last decade. Even if children are cured, they face lifelong challenges including cognitive and growth delay and the development of secondary malignancy. Our laboratory is working on understanding RMS biology because it is unknown which targets should be shut down by drug therapy to eliminate this tumor. The overall goal of our project is:

1. to determine if RMS models can be generated to resemble human cancer as closely as possible
2. to use these models to better understand how the cancer is being formed and
3. by understanding how the cancer arises, to develop more effective drug therapy that can be tested in our models and ultimately used to successfully treat RMS in children.

Dr. Gladdy is a Surgeon Scientist and Assistant Professor at the University of Toronto who joined the faculty in 2008 after completing a Surgical Oncology Fellowship at Memorial Sloan-Kettering Cancer Center in New York. Her independent research program at the Lunenfeld-Tanenbaum Research Institute is focused on developing functional genomics platforms to inform the development of much needed effective therapies for sarcoma patients. Her clinical expertise is in the surgical management of soft tissue sarcoma. Dr. Gladdy is a member of the Sarcoma Program, which is the Centre of Excellence for Sarcoma Care in Canada and is affiliated with Princess Margaret Cancer Centre, Mount Sinai Hospital (MSH), and the Hospital for Sick Children. In addition to having a translational research lab, she is the Director of the GI Sarcoma Clinical Research Group at MSH, which is dedicated to advancing clinical care through engaging in clinical trials and improving quality of life for sarcoma patients

1:25-2:00 OVC LLC Room 1714

Dr. Urban Emmenegger

Clinician Scientist, Odette Cancer & Sunnybrook Research Institute, Department of Medicine, & Institute of Medical Science, University of Toronto

Stereotactic ablative radiotherapy (SABR) of oligometastatic prostate cancer: true clinical value or doing the feasible?

Metastatic prostate cancer is an incurable condition with limited life expectancy despite numerous therapeutic advances over the last few years. As of late, the first-line treatment of widely metastatic prostate cancer typically involves androgen deprivation combined with early docetaxel chemotherapy, an approach that improves survival by >1 year compared to androgen deprivation alone. However, the survival benefit of this strategy remains to be determined in patients with low metastatic burden, including men with *de novo* oligometastatic prostate cancer. The latter is a state between loco-regional and widespread metastatic disease, defined by ≤ 5 metastatic lesions. Aside from its *de novo* existence oligometastatic prostate cancer can be induced by effective systemic therapy, or it may present as oligoprogression (i.e. a clinical situation where ≤ 5 metastatic tumor sites progress, while all other metastases are controlled by a given systemic therapy). SABR is very intense and focused external-beam radiotherapy used to eradicate primary tumors and metastases with high precision and relatively mild toxicity. As such, SABR is increasingly considered for the treatment of men with oligometastatic prostate cancer to delay the initiation of systemic therapy or of the next line of treatment, and to possibly improve survival. However, there is a lack of high-level randomized outcome data, and there are no markers predicting patients that derive only limited benefit from SABR of oligometastases because of early and widespread metastatic progression following treatment. In fact, it remains to be seen if oligometastatic versus widely metastatic malignancies are distinct biological entities. I will discuss some of the challenges of this novel treatment paradigm by summarizing current research activities of SABR for different stages of metastatic prostate cancer at Sunnybrook Odette Cancer Centre.

Dr. Emmenegger obtained his medical degree from the University of Bern/Switzerland in 1992. Following training in Internal Medicine and Medical Oncology, from 2001 to 2008 he joined the laboratory of Dr. Robert S. Kerbel (Sunnybrook Research Institute) to undertake postdoctoral training in tumor biology, with special emphasis on tumor angiogenesis and metronomic chemotherapy. He also completed a fellowship in Medical Oncology at the Odette Cancer Centre from 2005 to 2008. Dr. Emmenegger is specialized in the systemic treatment of advanced prostate cancer. Aside from clinical trial activities, he is interested in characterizing relevant drug - drug interactions between novel prostate cancer medications and medications frequently used by the typically elderly prostate cancer patient with numerous co - morbidities. His laboratory team aims to improve metronomic chemotherapy by characterizing mechanisms of resistance, and by identifying predictive markers of response. Additional studies focus on how fibroblast growth factor 23 contributes to aggressive prostate cancer behaviour.

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

Morning Session

Effect of exercise and n-3 polyunsaturated fatty acids on mammary tumour development in mouse model of human breast cancer

Zhen Lin¹, Daniel Pyc¹, Lyn Hillyer¹, William Muller², Graham Holloway¹, Lindsay Robinson¹, David W.L. Ma^{1*}

¹Department of Human Health & Nutritional Sciences, CBS, University of Guelph; ²Oncology Labs, McGill University, Montreal

Introduction. Breast cancer risk has been shown to be modifiable through lifestyle factors including diet and exercise. The inhibitory effects of n-3 polyunsaturated fatty acids (PUFA) on mammary tumour development have been previously demonstrated in human and animal studies. Similarly, physical activity has been shown to beneficially influence breast cancer outcomes in human and animal studies. However, previous studies have not explored the combined effects of life-long exposure to dietary n-3 PUFA and aerobic exercise on breast cancer outcomes. Therefore, the objective of this study was to investigate the influence of dietary n-3 PUFA and aerobic exercise on mammary tumourigenesis in the mouse mammary tumour virus (MMTV)-neu(ntl)-YD5 (MMTV-neu) breast cancer model. Methods. Heterozygous MMTV-neu males were bred with wild type FVB female mice generating heterozygous MMTV-neu females offspring which were placed on one of two diets, 10% safflower (n-6 PUFA) or 3% menhaden plus 7% safflower (n-3 PUFA), then randomized to remain sedentary or receive a treadmill exercise protocol. From 8 weeks of age, female progeny received a 4-week exercise intervention consisting of daily 45 minute treadmill running, 5 days per week. Results. Findings suggest that regardless of exercise, n-3 PUFA fed mice had reduced mammary tumour size and multiplicity relative to n-6 PUFA fed mice. Exercise delayed the development of tumours when examining the percent of mice tumour free in both diet groups. Conclusion. The present study provides evidence for a beneficial combinatory effect of diet and exercise on mammary tumour development. Translated to humans, this suggests that lifestyle affects the quality of life by delaying mammary tumour development.

Funding: Cancer Research Society

Analysis of Munc18c and Syntaxin4 Function During Tumor Cell Invasion in MDA-MB-231 Cells

Megan Brasher, David Martynowicz, Marc Coppolino*

Department of Molecular & Cellular Biology, CBS, University of Guelph

The ability of cells to invade and migrate through the extracellular matrix (ECM) is an essential component of many physiological and pathological processes. Similar cellular processes that mediate invasion are also responsible for the progression of pathological disorders. When the molecular interactions that control invasion in the ECM are altered, the results can lead to diseases such as cancer. Degradation of the ECM facilitates the spread of cells to secondary sites and is characteristic of invasive cancer cells. In tumor cells, invasion can occur through actin-

driven cell membrane protrusions, known as invadopodia, which utilize both cell surface ECM receptors and proteolytic enzymes for their formation and function. Membrane trafficking of proteins to invadopodia is required for their formation. Key proteins required during intracellular membrane trafficking events are SNAREs, which function to localize and fuse vesicles with target membranes. Previous studies have shown that the SNARE Syntaxin4 is involved in the formation of invadopodia in MDA-MB-231 cells, however it is unclear how Syntaxin4 function is regulated during tumor cell invasion. Munc18c, a Sec1/Munc-like (SM) protein, is a known regulator of Syntaxin4 activity. Previous work in our lab has shown a potential role for Munc18c in invadopodium based ECM degradation and cell migration. It has been found that knockdown of Munc18c perturbs invadopodia formation, as well as cell migration. The inhibition of Munc18c function produced similar results to knockdown experiments. Munc18c inhibition also causes decreased interaction between the SNAREs Syntaxin4 and SNAP23. These results suggest Munc18c facilitates Syntaxin4 function during tumour cell invasion.

Funding: NSERC

Hypoxia represses E-cadherin translation and activates cadherin 22 synthesis via eIF4E2 to drive the migration, invasion and adhesion of breast cancer cells.

Nicole Kelly, James Uniacke*

Department of Molecular & Cellular Biology, CBS, University of Guelph

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, causes the suppression of the standard protein synthesis machinery to limit energy expenditure. Hypoxic cells switch from utilizing eukaryotic initiation factor 4E (eIF4E) to eIF4E2 to initiate the selective translation of certain mRNAs. Cancer cells exploit this pathway for their growth, proliferation, and survival. One target of eIF4E2 includes the cell-cell adhesion molecule, cadherin 22. This molecule is upregulated by cells during hypoxia and may allow cancer cells to adhere to one another in the hypoxic core of tumors. Cadherin 22 may also be involved in the collective cell migration of cancer cells, which promotes metastasis and immune evasion. By characterizing the role of eIF4E2 and cadherin 22 in cancer, this project will enhance our understanding of tumor biology and offer an interesting avenue for future therapeutic interventions.

Funding: Canadian Cancer Society, Cancer Research Society, CFI

Investigating the molecular signaling mechanisms of the neuronal adaptor protein, ShcD, in development and cancer

Hayley Lau, Melanie Wills, Ava Keyvani Chahi, Nina Jones*

Department of Molecular and Cellular Biology, CBS, University of Guelph

The Shc family of PTB/SH2 domain-containing adaptor proteins serves to connect upstream phosphotyrosine-based inputs with downstream regulation of key cellular effectors such as Erk/MAPK. Our laboratory has previously shown that ShcD, the most newly discovered

member, is overexpressed in human gliomas, and that it associates with several neuronal receptor tyrosine kinases, including TrkA, TrkB and Ret. We now reveal that ShcD represses Erk activation downstream of these oncogenic receptors. ShcD binds the activated receptors via its PTB and SH2 domains, where it becomes phosphorylated on central tyrosine residues through receptor-mediated phosphorylation. Heightened ShcD phosphorylation is associated with reduced Erk activation, and we show that ShcD is repressing Erk activation downstream of TrkB by indirectly preventing Grb2 from interacting with the receptor. In addition, we uncover the importance of the central phosphotyrosine residues found on ShcD, in the binding and sequestering of Grb2 away from TrkB. Lastly, we confirm that systemic loss of ShcD in mice leads to increased neuronal Erk activity. Activation of Erk is a key step in neuronal differentiation, thus we propose that this novel molecular mechanism could contribute to the undifferentiated nature of gliomas harbouring increased ShcD expression.

Funding: NSERC

Colorectal cancer cells resistant to the chemotherapeutic agent SN-38 gain integrin $\alpha 5 \beta 1$ expression and signalling

Spencer IT Berg¹, Murray J. Cutler¹, Jonathan Blay^{1,2*}

¹School of Pharmacy, University of Waterloo, Waterloo, ON; ²Department of Pathology, Dalhousie University, Halifax, NS

Management of advanced colorectal cancer (CRC) includes chemotherapy as an essential part of the treatment regimen, however for many patients chemotherapy fails. The ensuing relapse is often more aggressive than the original disease, and thus these patients face a poor prognosis. Cancer cell behaviour is strongly influenced by the tumour microenvironment in many ways, including response to chemotherapy. We therefore chose to study how an SN-38-resistant CRC cell line, HT29-S, differed from its chemotherapy-sensitive counterparts, HT29, in its interaction with a component of the tumour microenvironment, the extracellular matrix (ECM). We discovered that HT29-S cells strongly upregulated the $\alpha 5$ integrin subunit, and gain the ability to adhere to and construct matrices of the ECM protein fibronectin, which is the natural ligand of the $\alpha 5 \beta 1$ heterodimer. Furthermore, HT29-S but not HT29 cells demonstrated features associated with an active integrin $\alpha 5 \beta 1$ -fibronectin signaling axis, including resistance to extended serum deprivation, phosphorylation of protein kinase B (Akt), and upregulated expression of the anti-apoptotic B-cell lymphoma 2 (BCL2) gene. Interfering with phosphoinositide 3-kinase (PI3K)/Akt signalling with the small molecule inhibitor LY294002 partially restored the sensitivity of HT-29S cells to SN-38 but did not change the response of parental HT-29 cells to the drug. This data suggests a contribution of integrin $\alpha 5 \beta 1$ to the resistant status of HT29-S, and provides support for the development and targeted use of integrin $\alpha 5$ inhibitors.

Funding: NSERC

Prognostic value of activated Smad2 and TAZ levels in appendicular canine osteosarcoma: a pilot immunohistochemistry study in a tissue microarray

Anita Luu¹, Courtney Schott², Geoffrey Wood², Alicia Vilorio-Petit^{1*}

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

Canine osteosarcoma (OSA) is a commonly diagnosed and aggressive bone tumour that lacks reliable molecular prognostic markers. Transforming growth factor beta (TGF β) and transcriptional co-activator with a PDZ-binding motif (TAZ) are cooperative mediators of bone development and cancer progression. Interestingly, their prognostic value in canine OSA is rather unexplored. This pilot study employed a tissue microarray comprised of 31 appendicular primary canine OSA tumour samples, to examine the levels of phosphorylated Smad2 (pSmad2), key effector of classical TGF β signalling, and TAZ by immunohistochemistry. Markers were scored using the Allred method. Fisher's exact test was used to seek associations between pSmad2 or TAZ levels, and tumour grade or alkaline phosphatase (ALP) status. Associations between marker levels and metastasis or overall survival (OS) were evaluated using Kaplan-Meier plots and the log-rank test. Neither pSmad2 nor TAZ levels were significantly associated with tumour grade, ALP status, and time to metastasis or OS. However, patients with high pSmad2 or TAZ levels had a shorter median time to metastasis (2.65 times shorter, HR=1.562 for pSmad2 and 2.95 times shorter, HR=2.157 for TAZ, respectively). Patients with high levels of both markers had a 6.8 times shorter median time to metastasis, and 1.7 times shorter OS. The trend toward reduced time to metastasis in patients with high levels of each marker alone or in combination was more evident in patients treated with amputation and carboplatin. Immunohistochemical determination of TAZ and pSmad2 levels has potential prognostic value in canine OSA. Further analyses in larger patient cohorts are warranted.

Funding: OVC Pet Trust Fund

Fever-Grade Temperatures Attenuate Rhabdovirus-Mediated Oncolysis of Cancer Cells

Jacob P. van Vloten¹, Mary Ellen Clark¹, Katrina Geronimo², Arthane Kodeeswaran², Lisa Santry¹, Rob C. Mould¹, Grant McFadden³, James J. Petrik⁴, Sarah K. Wootton¹ and Byram W. Bridle^{1*}

¹Pathobiology & ⁴Biomedical Sciences, OVC, University of Guelph; ²St. Joan of Arc Catholic Secondary School, Mississauga, Ontario; ³Molecular Genetics & Microbiology, University of Florida, Gainesville, Florida, USA

Oncolytic viruses (OVs), including vesicular stomatitis virus (VSV), maraba virus (MG1), Newcastle disease virus (NDV) and orf virus (ORFV) are promising agents for cancer immunotherapy. They lyse cancer cells, release tumor antigens and stimulate adaptive anti-tumor immune responses. VSV, MG1 and NDV have been undergoing testing in humans and companion animals. When administered to patients, one side effect is induction of fever. Traditionally, in vitro assays test the efficacy of OV's at normal human body temperature (37°C) and therefore do not provide information on fever conditions, nor companion animals that have higher body temperatures (~39°C). We investigated the effect of elevated temperatures on OV-mediated oncolysis. Human, murine, feline and canine cancer cell lines were used to represent a range of species and cancer cell types relevant to clinical and pre-clinical models. Resazurin assays were conducted at 37, 38.5 and 40°C in each cell line. Surprisingly,

at elevated temperatures, the oncolytic potential of the rhabdoviruses VSV and MG1 was severely attenuated or completely abrogated in all cell lines. In contrast, neither NDV nor ORFV oncolysis was compromised, suggesting this phenomenon is partially virus-dependent. Interestingly, NDV-mediated oncolysis was often potentiated at higher temperatures. Growth curve assays at temperatures increasing from 37°C to 40°C demonstrated a temperature-dependent attenuation of MG1, beginning at 38.5°C. These results demonstrate the importance of elevated temperature with respect to OVIs and have implications for managing temperatures of human patients, applications in companion animals and interpretation of safety studies in non-human primates, which have normal body temperatures of ~39°C.

Funding: Cancer Research Society; NSERC; OVC Scholarship

Afternoon Session

ICCI comparative oncology program: Utilizing spontaneous companion animal cancers as models for human cancers

Vicky Sabine¹, Kaya Skowronski¹, Michelle Oblak¹, Geoff Wood², Brenda Coomber³, Paul Woods^{1*}

¹Clinical Studies, ²Pathobiology, ³Biomedical Sciences; OVC, University of Guelph

Similar to people, cancer is common in companion animals with approximately 1 in 3 dogs and 1 in 7 cats developing cancer and ~50% of pets over 10 years old dying of the disease. In addition, many of the cancers of pets share the same characteristics as cancer in people. Oncology-related clinical research trials at OVC HSC are performed with the capability of the Institute for Comparative Cancer Investigation (ICCI). The ICCI combines expertise in basic cancer biology and veterinary medicine enabling an integrated approach to cancer studies that cannot always be matched in a human research setting. The ICCI is the only Canadian centre involved in the National Institute of Health-National Cancer Institute (NIH-NCI) Comparative Oncology Trials Consortium (COTC). Both ICCI and COTC complement translational research by utilizing naturally occurring spontaneous cancers that develop in companion animals to facilitate the improvement of healthcare for animals and also provide robust clinical data relevant for future human clinical trials. Currently at OVC HSC, there are 14 oncology-related clinical trials actively recruiting patients: 9 canine, 2 feline and 3 both cats & dogs (<http://ovc.uoguelph.ca/icci/trials>). In particular, the ICCI's first collaboration with the NIH-NCI-COTC recently started with an investigation of an mTOR inhibitor (rapamycin) in addition to the standard of care therapy in dogs with osteosarcoma. Hence, the ICCI comparative oncology program at the OVC HSC has the potential to improve the lives of both animals and people.

Funding: Smiling Blue Skies Cancer Fund & OVC Pet Trust Fund

Vitamin D status and acute phase protein concentrations in canine cancer patients

Weidner N¹, Woods JP², Mutsaers AJ^{1,2}, Bayle J³, Verbrugghe A^{2*}

Departments of ¹Biomedical Sciences, ²Clinical Studies, OVC, University of Guelph; ³Royal Canin Research Center, Aimargues, France

Lower blood 25-hydroxyvitamin D (25(OH)D) levels and increased acute phase protein (APP) concentrations, have been observed in canine cancer patients. Links between these variables have been observed in humans, but have not been studied in canines. Our objective was to determine the relationship between blood 25(OH)D and APP in healthy dogs and dogs with cancer. Client-owned dogs with lymphoma (n=34), osteosarcoma (n=21), mast cell tumors (MCT) (n=26), and healthy dogs (n=25), were enrolled. Blood samples, collected before treatment, were analyzed for plasma 25(OH)D using radioimmunoassays, and plasma C-reactive protein (CRP), haptoglobin (Hp), alpha-1-acid glycoprotein (AAG) and serum amyloid A (SAA) using ELISA. Variance analyses with adjusted post-hoc analysis were parametrically performed or not as appropriate. Pearson correlation coefficients were also calculated. Lymphoma patients showed higher circulating CRP, Hp, SAA, AAG concentrations and lower 25(OH)D concentrations than healthy dogs (p<0.001, p<0.001, p=0.010, p=0.004, p=0.035). Osteosarcoma dogs had elevated blood CRP, Hp, and SAA concentrations (p<0.001, p=0.005, p=0.010) and MCT dogs had increased Hp concentrations (p=0.020) compared to healthy dogs. Correlations were found between plasma 25(OH)D and AAG concentrations (r=-0.48, p=0.004) in lymphoma patients, and 25(OH)D and CRP in MCT patients (r=0.41, p=0.040), and 25(OH)D and SAA in MCT patients (r=0.43, p=0.027). 25(OH)D and APP concentrations are consistent with previous findings, although this is the first report for some of these APPs pairings and cancers in dogs. Certain APP concentrations in dogs with cancer were above reference range. Future research investigating the influence of cancer treatment on these variables is warranted.

Funding: OVC Pet Trust Fund, AAVN-Waltham Research Grant, Royal Canin

Comparison of a ventral cervical versus two lateral incisions for extirpation of the mandibular and medial retropharyngeal lymph nodes

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Department of Clinical Studies, OVC, University of Guelph

Two techniques have been previously described to perform bilateral mandibular and medial retropharyngeal (MRLN) lymphadenectomy for staging of oropharyngeal tumors in dogs. In this randomized cross-over cadaver study we compared the two described techniques and reported time, size of incision and operator satisfaction with each of the described techniques. Sixteen canine fresh-frozen cadavers were obtained and eight veterinarians with advanced surgical training were randomized to perform both techniques sequentially. Time to extirpation of lymph nodes, successful removal and overall incision length was recorded. Following both procedures, participants rated their experience with each technique and provided preferences between the techniques for identification of lymph nodes and visualization of local anatomy. Data was analyzed using a Wilcoxon rank sum test and paired t-test. Incision length was the only significant finding with the lateralized incisions being significantly longer than the ventral cervical technique (p=0.0027). No significant difference was found between techniques for time

to removal of the first and last lymph node. Limitations of this study included a small sample size and potential bias due to previous experience. Overall preferred technique by participants was the ventral cervical approach, due to its perceived superior visualization of local anatomy. Although overall time for removal of lymph nodes was not different between both techniques evaluated, a difference in incision length correlates to a longer closure time and thus overall surgery time. The perceived improvement of identifying local anatomy by the ventral midline approach could potentially translate to improved identification of lymph nodes and reduced morbidity.

Funding: none

RNA Disruption as an indicator of CHOP therapy efficacy in canine lymphoma

Laura B Pritzker^{1*}, Baoqing Guo¹, Rashmi Narendrula¹, Shirly Wang¹, Lin Laura Lin¹, Karolina Skowronski², Dorothee Bienzle³, J Paul Woods², Kenneth P H Pritzker¹, Amadeo M Parissenti^{1,5}, Brenda L Coomber⁴

¹Rna Diagnostics, Toronto; ²Clinical Studies, ³Pathobiology & ⁴Biomedical Sciences, OVC, University of Guelph; ⁵AMRIC & Laurentian University, Sudbury, ON

The RNA Disruption Assay (RDA) was developed based on findings from a human breast cancer clinical trial; mid-therapy tumour samples from patients undergoing chemotherapy were found to have RNA fragmentation (RNA disruption) which correlated with subsequent pathological complete response (pCR). Further investigation into the RNA electropherogram patterns led to the creation of an RNA Disruption Index (RDI), which specifically measures chemotherapy-induced RNA fragmentation. In order to expand our understanding, we investigated RNA Disruption as a predictor of remission duration in canine lymphoma. CHOP therapy is standard of care for dogs that present with lymphoma; however, 60% of these dogs do not achieve a long lasting remission. RNA Disruption was measured during the first round of CHOP therapy using fine needle aspirate (FNA) samples taken at four time points (0, 14, 28 and 56 days) to assess how early in treatment this effect can be measured and how RDI values change over time. Forty-one dogs were enrolled in the study and FNA sample adequacy rate was 78% early in therapy and decreased to 62% after 7 weeks. A significant difference was found in both maximum and average RDI values between dogs which had a complete response and those who had progressive disease at 6 months. In addition, both average RDI and maximum RDI significantly correlated with the overall survival. This study has given insight into the process of RNA disruption as an outcome of chemotherapy and has provided further evidence supporting the use of RDA as a clinical diagnostic tool.

Funding: OVC Pet Trust Fund, Rna Diagnostics Inc.

Novel oncolytic Maraba virus for the adjuvant treatment of feline mammary carcinoma

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Feline mammary carcinomas (FMC) are highly metastatic tumours. Surgery has been the treatment of choice; however, the median disease free survival following surgery is 390 and 240 days for grade 2 and 3 carcinomas respectively. Adjuvant chemotherapy has not demonstrated benefit. The purpose of this study was to employ an adjuvant oncolytic Maraba virus vaccine following surgery for FMC. Cats were staged, vaccinated with an adenovirus expressing a cancer gene, 2 weeks later the tumour was excised, and 4 weeks post surgery the cats received a booster with an oncolytic Maraba virus also expressing the cancer gene. Cats were followed up with physical exams and thoracic radiographs. Twelve female (spayed) cats have entered the study with a mean age of 12.5 years (range 7.5-16.8), median weight of 4.4 kg (range 3.25-5.89), consisting of 7 DSH, 4 DLH, 1 Turkish Van. The FMC consisted of 5 grade 2, 6 grade 3; 7 stage 1, 1 stage 2, 4 stage 3. One cat died of putative anaphylaxis during infusion of the oncolytic virus prompting introduction of screening for pre-existing hypersensitivity. Otherwise, toxicity has been manageable. Six cats have died of disease. Five cats are still alive (ranging from 92 - 568 days). The median overall survival is 240 days. Except for the one catastrophic anaphylactic reaction, the treatment has been well tolerated. Employing a novel oncolytic virus to implement the heterologous prime:boost strategy to target tumour antigens may result in a new modality for the adjuvant treatment of FMC following surgical excision.

Funding: Canadian Breast Cancer Foundation

LUNCH & POSTER SESSION 12:00-1:25

OVC LLC Room 1707 B & C

Posters will be displayed all day; authors please attend your poster 12:30-1:00
POSTER ABSTRACTS (*study leader)

1) Analysis of the TGF β -TAZ Signalling Network in Canine Osteosarcoma in Response to Doxorubicin Treatment

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Osteosarcoma (OSA) is the most common bone tumor in dogs, where it commonly metastasizes to the lungs. Metastatic canine OSA is resistant to chemotherapy and responsible for patient mortality. OSA metastasis and chemoresistance mechanisms are generally unknown. Transforming growth factor- β (TGF β) is a highly conserved cytokine with roles in bone development, bone disorders, cancer metastasis and chemoresistance. TGF β signalling is carried out through 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, differentiation, as well as bone mass and osteoblastogenesis. TAZ was shown to mediate self-renewal capacity and cancer metastasis. We hypothesized that the TGF β -TAZ signalling axis mediates OSA progression and chemoresistance. We first established the functionality of TGF- β signalling in a panel of canine OSA cell lines generated in house. The pattern of Smad2 activation and TAZ expression was determined using immunoblotting. Stemness marker gene expression was determined using qPCR. 24-hour treated cells were also examined for their colony-forming ability after doxorubicin treatment. All cell lines expressed TGF β receptors and showed a robust TGF β 1 response, displaying dose-dependent Smad activation that varied with time and cell line. 24-hour TGF β 1 treatment caused a dose-dependent TAZ increase in the majority of cell lines, and impacted their ability to form colonies after doxorubicin treatment. Further validation of these findings, and of approaches to target this axis, may lead to improved canine OSA therapy.

Funding: OVC Fellowship & OVC Pet Trust Fund

2) Mechanisms that allow oncolytic viral replication inside a tumour despite pre-existing immunity against a virus-encoded antigen

Wing Ka Amanda AuYeung¹, Robert C. Mould¹, J. Paul Woods², Geoffrey Wood¹, James Petrik³ and Byram W. Bridle^{1*}

¹Pathobiology, ²Clinical Studies, and ³Biomedical Sciences, OVC, University of Guelph

Oncolytic viruses (OVs) selectively target and destroy cancerous cells, while leaving normal cells unharmed. In addition to directly reducing tumour burden, OVs engineered as vaccine vectors can boost tumour-specific immune responses. We formally tested and confirmed the hypothesis that OVs are safer when used as booster vaccines. However, a mystery remained: How could OVs replicate and mediate oncolysis in hosts with pre-existing T cells specific for a tumour antigen that was also encoded by the virus? We hypothesize that this phenomenon was due to: (1) Acute virus-induced lymphopenia that transiently reduced transgene-specific tumour-

infiltrating lymphocytes (TILs), and/or (2) The immunosuppressive tumour microenvironment rendered TILs dysfunctional and inefficient at clearance of viruses. We used an intradermal B16-F10 melanoma model in which mice were primed with an adenovirus expressing the melanoma antigen dopachrome tautomerase (DCT) and boosted with vesicular stomatitis virus also encoding DCT. Our data suggest both proposed mechanisms provide a window of opportunity for OV's to replicate within a tumour, despite pre-existing virus-specific T cells. Specifically, the numbers of DCT-specific T cells inside tumours were reduced at six hours post-boost. Assessments of transgene-specific CD8⁺ TILs revealed impaired degranulation and reduced production of multiple cytokines, suggesting they were functionally impaired compared to blood- and spleen-derived T cells. Our results identify two mechanisms that allow virus-mediated oncolysis despite pre-existing virus-specific immunity. Although seemingly counterintuitive, this research identifies induction of OV-specific immunity as a strategy to enhance the safety of oncolytic virotherapy while retaining direct oncolytic activity.

Funding: BioCanRx, OVC Pet Trust Fund Scholar's program, NSERC CGS-M

3) Analysis of RREB-1/hnt function in *Drosophila* intestinal stem cells

Brittany Baechler, Cameron McKnight, Porsha Pruchnicki, Nicole Biro, Donya Saghattchi, Bruce H. Reed*

Department of Biology, University of Waterloo

The *Drosophila* gene *hindsight* (*hnt*), homologue of mammalian Ras responsive element binding protein-1 (RREB-1), encodes a C2H2-type Zinc finger nuclear protein that is required for several developmental processes including germ band retraction, tracheal differentiation, retinal morphogenesis, and proper growth of the follicular epithelium of the ovary. In some processes *hnt* has been identified as a direct target of the Notch signalling pathway. Given the importance of Notch signalling in the regulation of the adult intestinal stem cells (ISCs), we have investigated the regulation and function of *hnt* in this context. We show that *hnt* is expressed throughout the larval and adult midgut, and we further demonstrate that *Hnt* is required for ISC establishment during the larval/pupal transition. In the adult midgut we find that ISC *hnt* expression does not require Notch signalling, but is dependent on the EGFR/RAS/MAPK signalling pathway. Moreover, we find *Hnt* overexpression to be a potent effector of ISC loss through enterocyte (EC) differentiation. We also show that EC differentiation via *Hnt* overexpression can suppress ISC tumour formation associated with either the loss of Notch signalling or the activation of the EGFR/RAS/MAPK signalling pathway.

Funding: NSERC

4) Investigating the effects of hypoxia on the regulation of ribosomal protein gene expression and alternative splicing events

Andrea Brumwell, Jim Uniacke*

Department of Molecular & Cellular Biology, OVC, University of Guelph

The ribosome, a complex and highly conserved molecular machine, is widely considered unchanging in composition due to its critical role in translation. Recent observations of dysregulated ribosomal protein expression and rRNA modifications in diseases such as cancer are challenging this idea of the ribosome as a “static entity”. Specialized ribosomes have been observed in stressed yeast and bacterial cells, in which an altered ribosomal composition permits the translation of specific transcripts. Hypoxia, or low oxygen, is an important cell stressor that is involved in embryogenesis, ischemia, and cancer. Translation is significantly altered in hypoxia, such as the switch from eIF4E cap-binding protein to the homolog eIF4E2 in hypoxic translation initiation. I propose that human ribosomal protein gene expression and splicing is altered in hypoxia as an adaptation to cellular stress. We carried out a global expression analysis of 88 ribosomal protein genes, half of which changed by more than 20% in hypoxia. RPSA and RPL37 were chosen to study in detail due to their potential roles in cancer progression, often a hypoxia-driven process. I will focus on the roles of RPSA and RPL37 in the hypoxic translational apparatus, as well as their roles in cancer cell migration and p53 stabilization, respectively. We also measured 68 ribosomal protein gene alternative splicing events and select events will be investigated as possible biomarkers of tumor hypoxia. This study will provide insight into the adaptations of cells to the hypoxic environment and could be beneficial in developing more effective cancer diagnoses and therapeutic treatments.

Funding: OGS, NSERC

5) Inhibition of AKT-1 for the treatment of non-small cell lung cancer (NSCLC)

Paige Chorner, Roger Moorehead*

Department of Biomedical Sciences at the University of Guelph

Akt is a serine-threonine kinase implicated in tumorigenesis as a central regulator of cellular growth, proliferation, survival, metabolism, and migration. Activated Akt is overexpressed in approximately 55% of lung cancer tumors and has exhibited an association with poor prognosis as well as chemotherapeutic resistance to platinum-based therapy. Akt inhibitors such as MK-2206 are currently undergoing clinical investigation for the treatment of NSCLC however, these agents broadly target all three (1-3) Akt isoforms. The functional roles of the Akt isoforms were believed to be largely redundant but our lab has demonstrated that loss of Akt1 in transgenic mouse models inhibits while loss of Akt2 enhances lung tumor development. Therefore, it's hypothesized that preferentially inhibiting Akt1 will warrant a more effective therapeutic strategy compared to broad Akt inhibition. WST-1 cell viability assays have revealed that a selective Akt1 inhibitor A-674563 is a more potent regulator of survival in NSCLC cell lines compared to the pan-Akt inhibitor MK-2206. Immunofluorescence and western blot analysis have uncovered divergent downstream effects of the two drugs which may explain the therapeutic advantages of the Akt1 specific inhibitor. We hope to continue our investigation by confirming the specificity of the drugs by probing for isoform-specific targets and comparing our results to NSCLC cell lines with CRISPR/Cas9 knockouts of Akt1 and Akt2. We also hope to compare the effects of the two treatments in vivo on our transgenic lung cancer mouse models with overexpressed IGF-IR.

Funding: Canadian Cancer Society; OGS

6) Investigation the role of phosphotyrosine adaptor ShcD in EGFR- driven glioblastoma multiforme (GBM)

Maedeh Darzianiazizi, Melanie K. B. Wills, and Nina Jones*

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With mean survival rate of almost 15 months following diagnosis, glioblastoma multiforme (GBM) is the most common and lethal malignant primary brain tumor. Overall, aberrant amplification, overexpression, and mutation of wild-type (wt) epidermal growth factor receptor tyrosine kinase (EGFR) have been found in ~60% of GBM patients. In GBM, EGFR activation promotes cellular proliferation through activation of Ras (rat sarcoma) /MAPK (mitogen-activated protein kinase) and PI3K–Akt signaling pathways. Src homology and collagen (Shc) proteins, consisting of four unique members, ShcA, B, C and D, have been considered archetypal adaptors of EGFR–mediated signaling. Using quantitative PCR (qPCR) analysis we have recently detected high levels (a mean 41-fold increase) of ShcD expression in a selection of grade I–IV human brain tumors (gliomas), compared to benign brain tissues. The elevated ShcD expression was also coincident with EGFR hyperphosphorylation, which indicate the relevance of ShcD up-regulation and EGFR activation under pathological circumstances. While the clinical significance of EGFR deregulation is well documented in high-grade tumors of nervous system, herein, we aim to investigate the role of ShcD adaptor in aberrant EGFR signaling in GBM context through relevant cell- and mouse-based approaches. To our knowledge, ShcD is the first adaptor protein found to participate in aberrant EGFR signaling, which render it a novel and enigmatic component of the signal transduction machinery. By integrating the data from cell models and genetic mouse models, we might be able to develop a broader perspective on the role of ShcD both at cell and organism level.

Funding: OGS-OTS

7) Colorectal cancer cells resistant to SN-38 show elevated binding to fibronectin and localize within hepatic epithelial cultures

Heather Dekker¹, Spencer I. T. Berg¹, Murray J. Cutler¹, Jonathan Blay^{1,2*}

¹School of Pharmacy, University of Waterloo, Waterloo; ²Department of Pathology, Dalhousie University, Halifax

In colorectal cancer (CRC), chemotherapy may not be fully successful in eradicating disease. The surviving cells acquire resistance to the action of one or more of the drugs used in the initial treatment, and also change in other ways in their behavioural phenotype. We have previously shown that CRC cells made resistant to SN-38, the active metabolite of the drug irinotecan, have up-regulation of the integrin $\alpha_5\beta_1$ and accentuated activity of the $\alpha_5\beta_1$ signaling axis. In this work we have explored how this may affect their potential interaction with the extracellular matrix (ECM). We compared the adhesion properties of SN-38-resistant HT29-S cells with those of their chemotherapy-sensitive parent line HT29, in the context of both simple and complex ECM. When tested against substrata coated with fibronectin, HT29-S showed greater adherence than HT29 cells, consistent with up-regulation of $\alpha_5\beta_1$, which is the primary receptor for fibronectin. The major site for CRC metastasis is the liver, the ECM of which contains fibronectin. We used dense cultures of HepG2 cells as a model of the hepatic parenchyma to

study adhesion. Fluorescently-labeled HT29 and HT29-S cells adhered to HepG2 cultures with a differential localization that reflected the growth structure of the hepatic cell population, and with distinct features for the HT29-S cell line. Our findings therefore confirm enhanced adhesion of SN-38-drug-resistant CRC cells to fibronectin, and provide initial data suggesting that this may be relevant to the interaction of drug-resistant cells with the hepatic ECM in the formation of liver metastases in colorectal cancer.

Funding: NSERC

8) *Par6 links PI3K to the TGF β receptor complex: potential implications for PI3K targeting in advanced TGF β -dependent breast cancer*

Alham Delroba, Alicia M. Vilorio-Petit*

Department of Biomedical Sciences, University of Guelph

Transforming growth factor beta (TGF β) signaling promotes breast cancer metastasis, but also inhibits tumor growth during the early stages of cancer by promoting apoptosis. We previously observed that Par6, a key modulator of apical-basal polarity, is also a critical mediator of TGF β -induced apoptosis in early cancerous mammary cells. This requires Par6 phosphorylation by TGF β and correlates with a reduction in the activity of phosphatidylinositol 3-kinase (PI3K)/Akt. Based on this we hypothesize that a Par6-PI3K signaling axis functions within a cell polarity-associated signaling network that is crucial in determining cell death in response to TGF β , and propose that PI3K inhibition could potentially rescue TGF β -dependent apoptosis in advanced breast cancer cells. To address this, we first aimed at demonstrating a physical interaction between Par6 and PI3K. We performed Par6 and p85 (PI3K regulatory subunit) immunoprecipitation and immunoblotting on the lysates obtained from metastatic mammary cells over-expressing exogenous flag-tagged wild type Par6 or a dominant negative mutant Par6 that cannot be phosphorylated in response to TGF β . Our results indicate that Par6 and p85 interact within a complex that also include TGF β receptor I and the small GTPase Cdc42, and that this Par6-p85 interaction is constitutive. This was confirmed for endogenously expressed Par6 in two metastatic human breast cancer cell lines. Our ultimate goal is to use this knowledge to develop improved strategies to treat advanced breast cancers that rely on a functional TGF β -PI3K interaction for survival.

Funding: NSERC

9) *From Mice to Humans Via Dogs: Development of a Novel Biotherapy for Osteosarcomas*

Li Deng, Julia Kim, Robert C. Mould, Jacob Van Vloten, Amanda Au Yeung, Byram W. Bridle*

Department of Pathobiology, OVC, University of Guelph

Osteosarcomas (OSA) are the most common bone cancers in humans and dogs, with the latter having a 10 times higher incidence. We previously developed a cancer biotherapy, using a replication-deficient adenovirus and oncolytic rhabdovirus as heterologous prime-boost vaccines. To optimize the treatment for use in human OSA patients, it will be rigorously tested in OSA-

bearing dogs. Dogs spontaneously develop OSA, with remarkable similarities to the human disease. Our western blotting data confirmed observations made by others that survivin might be an ideal target antigen for OSA. We developed two recombinant viruses, human adenovirus serotype 48 (HAdV48) and maraba virus strain MG1 that carry transgenes encoding canine survivin. We have shown that HAdV48 can infect canine cells and mediate expression of a transgene; in this case, green fluorescent protein. A resazurin dye-based metabolic assay showed that canine OSA cells were sensitive to MG1-mediated oncolysis. Interestingly, dogs have higher body temperatures than humans. When investigating the effect of elevated temperatures on the oncolytic activity of MG1, 38.5°C was identified as a barrier to viral replication. This has implications for veterinary and human clinical trials; aggressive treatment of MG1-induced fevers may be beneficial and normal body temperatures <38.5 °C might be a potential inclusion criterion for canine trials. To monitor T cell responses during canine trials, a flow cytometry-based intracellular cytokine staining assay was optimized using blood-derived leukocytes stimulated with phorbol 12-myristate 13-acetate and ionomycin. A canine osteosarcoma trial has been designed and we are entering the vaccine production and release testing phases.

Funding: Terry Fox Research Institute

10) Prognostication of Canine T-cell Lymphoma

Nariman Deravi¹, Olaf Berke², Anthony Mutsaers³, Michelle Oblak³, Stefan Keller¹, Dorothee Bienzle^{1*},

Departments of ¹Pathobiology, ²Population Medicine, ³Clinical Studies, OVC, University of Guelph.

Lymphoma is a common cancer in dogs frequently treated with chemotherapy. Lymphoma is broadly grouped into B- or T-cell types through detection of CD79a or CD21 (B) and CD3 (T) markers. In general, T-cell lymphoma is considered to have a worse prognosis than B-cell lymphoma. However, there are different types of T-cell lymphoma with variable biological behaviour. Detection of additional markers such as CD4, CD5 and CD8 allows further classification of T-cell lymphomas. We hypothesized that specific T-cell lymphoma subtypes have distinct responses to chemotherapy and survival times. In this prospective study dogs with T-cell lymphoma evaluated at the OVC-HSC are enrolled Patients will be assessed according to standard of care. Enlarged lymph nodes will be aspirated for cytopathology and flow cytometry. The attending surgeon will excise an enlarged lymph node, which will be transected with one half fixed and sectioned for histopathology using standard protocols, and portions of the other half frozen in cryopreservation media. Sections will be assessed for architecture and cytomorphology, and by immunohistochemistry for expression and location of CD3 (T-cell), CD79a (B-cell), and granzyme B (cytotoxic T-cell). On frozen tissue CD4 (helper T-cell) and CD8 (cytotoxic T-cell) expression will be determined. Sections will be assessed and classified following World Health Organization guidelines, and results compared with other test results (flow cytometry and cytopathology), survival, and chemotherapy response. Nine patients have been recruited for within the first year, and include 6 dogs with large and 3 dogs with small T-cell lymphoma. Data collection and analysis are ongoing.

Funding: OVC Pet Trust Fund

11) Colorectal cancer cells made resistant to the irinotecan active metabolite SN-38 exhibit changes in cell-surface enzymes that are implicated in adenosine-dependent cell regulation

Bogdan Diaconu¹, Spencer I. T. Berg¹, Murray J. Cutler¹, Jonathan Blay^{1,2,*}

¹School of Pharmacy, University of Waterloo, Waterloo; ²Department of Pathology, Dalhousie University, Halifax

Systemic chemotherapy of most solid carcinomas is invariably accompanied by the gradual development of drug resistance if the therapy fails to eradicate the cancer. In addition, the nature of the cell population changes in further ways that favour cancer progression, such that the metastatic cancer may show both drug resistance and other alterations that permit its ongoing survival. We are modeling this process in cell culture with a range of colorectal cancer (CRC) cell lines in which we have induced the development of a drug-resistant phenotype. In this study, we have evaluated how certain aspects of the CRC cells change in parallel to the emergence of resistance to SN-38, the active metabolite of irinotecan (one of several drugs used in CRC chemotherapy). We examined the mRNA expression of the cell-surface proteins CD13, CD26, CD38, CD39 and CD73 in both HT-29 cells and their SN-38-resistant counterparts HT-29S cells. These proteins are a group of ecto-enzymes involved in the mediation of various nucleoside- and peptide-dependent pathways that participate in cell regulation. End-point RT-PCR analysis showed alterations in the expression of the mRNAs for CD38 and CD73 that correlated with the acquisition of the SN-38 drug resistant phenotype. CD38 and CD73 are involved in the generation of adenosine from adenine nucleotides in the extracellular milieu. Adenosine has multiple effects on cancer growth, survival and immune defence. These findings point to changes in the potential control of adenosine-dependent cell regulation as CRC carcinoma cells evolve or change in response to a chemotherapy regimen.

Funding: NSERC

12) Characterization of eIF4G3 and DDX28 as novel translation initiation factors in low oxygen conditions

Sonia Evagelou, James Uniacke*

Department of Molecular & Cellular Biology, CBS, University of Guelph

Ordinarily, translation is most prominently initiated by the formation of the eukaryotic translation initiation factor 4F (eIF4F) complex at the m⁷GTP cap structure located at an mRNAs 5' end. However, during times of low oxygen stress (hypoxia), a common feature of the tumor microenvironment, the canonical cellular translation machinery is inhibited as an adaptive mechanism to conserve energy supplies. Hypoxic cells are able to selectively synthesize thousands of proteins required for survival by switching from the use of the cap-binding subunit eIF4E, to its homolog eIF4E2. We hypothesize that eIF4E2 functions as part of a larger hypoxic translation initiation complex homologous to eIF4F that includes the eukaryotic translation initiation factor 4G3 (eIF4G3), and the DEAD-box protein DDX28. Our data from polysome profile analysis, co-immunoprecipitation, immunofluorescence and differential centrifugation, suggest a role for eIF4G3 in hypoxic translation and for DDX28 as an eIF4E2 binding partner that displays altered nuclear localization under hypoxia. Characterization of eIF4G3 and DDX28 as components of the hypoxic translation initiation complex, alongside eIF4E2, could aid in the

development of cancer therapeutics that specifically target the translational apparatus of cancer cells that reside in a tumors hypoxic core.

Funding: NSERC

13) *Investigating a role for Nck cytoskeletal adaptors in breast cancer development*

Adam Golding, Claire Martin, Nina Jones*

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Over 1.5 million women are diagnosed with breast cancer each year. Metastatic breast cancer is incurable with a 5-year survival rate of 23%; however, the cellular changes that allow the disease to become metastatic are poorly understood, limiting development of novel therapies. An unusual feature gained by metastatic breast cancer cells is invadopodia, which are cell surface structures that promote motility and degrade the extracellular matrix to promote invasion. The Nck cytoskeletal adaptor proteins (Nck1 and Nck2) are essential for invadopodia formation, through their direct ability to regulate and rearrange the actin cytoskeleton network. Nck was established as an oncogene over twenty years ago and recent findings suggest a correlation between Nck overexpression and enhanced invasion in melanoma; however, its role in breast cancer remains poorly understood. Preliminary expression profiling indicates that Nck1 is upregulated in breast cancer, with the highest expression in advanced stages. To further analyze the potential role of Nck misregulation in impacting both breast cancer formation and progression, I will use the MMTV-NIC transgenic mouse model of HER2-positive breast cancer. Parallel to this animal model, I will support my analysis by observing molecular and cellular effects of Nck gain and loss of function in a HER2-positive breast cancer cell line. Overall, I hope to gain insight into potential mechanisms by which Nck misregulation could promote cancerous cellular behavior, including invadopodia formation and metastasis.

Funding: Canada Research Chairs

14) *Glioblastoma cells cultured under physiological oxygen conditions have higher viability and lower reactive oxygen species*

Brianna Guild, Erin Specker, Jim Uniacke*

Department of Molecular & Cellular Biology, CBS, University of Guelph

Human cell culture is widely used as a model to investigate and characterize molecular and biochemical processes. However, oxygen availability, a crucial variable in human physiology, is often neglected in cell culture. Indeed, cell culture is typically performed in ambient air (21% oxygen), termed normoxia, while the documented physiological oxygenation range of human tissues, termed physioxia, is 1–11%. Several studies have suggested beneficial effects of culturing mammalian cells in low oxygen; however, to date there has been no systematic investigation of human cell cultures within the physioxic range. Here we show that culturing human primary glioblastoma cells within physioxia promotes cell viability and metabolic activity, decreased cellular reactive oxygen species levels and optimal mitochondria morphology.

Our data suggests that culturing human cells in physioxia may provide a better model for making physiological inferences.

Funding: NSERC

15) *Genomic Analysis of a Mouse Model for IGF-IR Driven Breast Cancer*

Rob Jones, Katrina Watson, Roger Moorehead*

Department of Biomedical Sciences, OVC, University of Guelph

Genetically engineered mice are valuable tools for understanding the genetic events that contribute to breast cancer development and progression. We have previously generated a doxycycline (Dox) inducible model of breast cancer, MTB-IGFIR, in which overexpression of the human type-I IGF receptor (IGF-IR) leads to the rapid induction of estrogen receptor negative (ER-) mammary tumors. Following Dox withdrawal and transgene downregulation, a subset of tumors escape IGF-IR dependence and spontaneously recur with evidence of an epithelial-mesenchymal transition (EMT). To gain insight into the molecular pathways that drive primary and recurrent tumor growth in MTB-IGFIR mice, we carried out gene expression profiling and DNA copy number analysis. Gene-set enrichment analysis (GSEA) revealed expression of an Akt oncogenic signature in primary tumors, consistent with downstream signaling mediated by IGF-IR. Interestingly, primary tumors also expressed a luminal progenitor gene signature suggesting a potential cell of origin. Recurrent tumors were found to express a mammary stem cell (MaSC) gene signature, which is consistent with their more mesenchymal phenotype as well as increased cell cycle pathway activity. While aCGH analysis identified few genomic changes in primary tumors, recurrent tumors exhibited gains and losses in known oncogenes and tumor suppressors including Met receptor, Yap1 and Cdkn2a. Integration of mRNA and copy number data confirmed altered expression of these genes at the transcriptional level. Taken together, these results suggest IGF-IR drives tumor initiation in part through activation of the Akt pathway and that multiple genetic alterations contribute to the acquisition of oncogene independence and tumor relapse.

Funding: CIHR, Canadian Cancer Society

16) *β -Parvin Mediates Novel Integrin-Cadherin Signaling Pathways During Tissue Morphogenesis*

Justin Knapp, Catherine Studholme*, Mungo Marsden

Department of Biology, University of Waterloo

Cell adhesion is a fundamental biological process in multi-cellular organisms. The dynamic regulation of cell adhesion is critical for the cell rearrangements that occur during both development and tumour metastasis. Integrin and cadherin receptors are key regulators of cell-extracellular matrix (ECM) and cell-cell adhesion respectively. *Xenopus* provides a simple model to examine the movements of cells in complex tissues and to elucidate the signaling pathways governing these rearrangements. Here we report that the integrin associated scaffolding protein β -parvin translocates between sites of integrin-fibronectin (FN) ligation and sites of

cell-cell adhesion. β -parvin consists of two calponin homology domains (CH1 and CH2). The CH1 domain is required for the translocation of β -parvin to sites of cell-cell adhesion and regulates C-Cadherin adhesion through the activation of Rac1. The CH2 domain on the other hand, regulates the association of β -parvin with integrin adhesion sites and regulates integrin adhesion through the activation of RhoA. We find that these local changes in cell behaviour mediated through β -parvin are responsible for cell migration, repulsion and cohesion during gastrulation. We demonstrate that β -parvin actively modulates cell adhesion via integrin-cadherin receptor cross talk and that this is essential for the cell rearrangements that drive early embryo morphogenesis.

Funding: NSERC

17) *Diets high in n-3 and n-6 PUFA differentially influence serum and mammary gland fatty acid composition in D6DKO mice*

Jessica MacKinnon, Salma Abdelmagid, David W. L. Ma*

Department of Human Health & Nutritional Sciences, OVC, University of Guelph

Background: Dietary n-3 and n-6 polyunsaturated fatty acids (PUFA) are thought to influence breast cancer development. This study investigated the individual effects these PUFA on mammary gland development and breast cancer risk by examining the effects of in utero vs. post-natal exposure to n-3 and n-6 PUFA on serum and mammary gland fatty acid composition, using the delta-6 desaturase knockout (D6DKO) mouse. Study Design: Harems were split into two groups, in utero (IU) and post-natal (PN). IU harems were maintained on diets containing 7% (w/w) from corn oil (LA), ARASCO (AA), menhaden oil (EPA/DHA), or flaxseed oil (ALA). Female offspring continued on this diet for 12 weeks after weaning. PN harems were fed a corn oil diet. Following weaning, female offspring were fed one of the experimental diets. Mice were terminated at 12 weeks. Fatty acid composition of mammary gland tissue and serum were determined by gas chromatography. Results: Preliminary work examined whether predicted fatty acids are found in serum and tissues of D6DKO mice. ANOVA of serum fatty acid composition confirmed knockout status of the mice and only precursor fatty acids were found in D6DKO mice. ANOVA also confirmed significant incorporation of dietary fatty acids into serum and mammary glands of mice maintained on their respective diets ($p < 0.05$).

Conclusions: These findings demonstrate that the D6DKO mouse model is suitable for elucidating the individual effects n-3 and n-6 PUFA. Future work will examine mechanisms of action related to gene and protein expression, cell signalling, cellular growth and apoptotic pathways.

Funding: Canadian Breast Cancer Foundation-Ontario Region Postdoctoral Fellowship, Public Health Agency of Canada, CIHR

18) *ISGylation is a Hypoxia-Induced Pathway that Attenuates Activity of the HIF-2 α Transcription Factor*

Gaelan Melanson, Jim Uniacke*

Department of Molecular & Cellular Biology, CBS, University of Guelph

The tumor microenvironment is characterized by chronic hypoxia (low oxygen tension) as cancer cells rapidly outgrow their blood supply. Many cancers take advantage of a gene program mediated by the transcription factors hypoxia-inducible factors (HIF) 1 α and 2 α that target genes containing hypoxic response elements (HREs) in their promoter region. These transcription factors play an important role in tumorigenesis by promoting vascularization through expression of angiogenic factors such as EPO and VEGF. Post-translational modifications offer a powerful tool to rapidly alter HIF-1 α and -2 α activities in response to hypoxia. Interferon-stimulated gene 15 (ISG15), a ubiquitin-like modifier, has been shown to down-regulate HIF-1 α activity in hypoxia, however, there is no information regarding the effects of ISGylation on HIF-2 α . HIF-2 α is a target of interest as it has been implicated in immune escape, and a more aggressive phenotype in developing tumors. Using the colorectal cancer model cell line HCT116, our data suggests ISG15 is a suppressor of HIF-2 α transcriptional activity. Overexpression of the ISGylation system enhanced HIF-2 α degradation in hypoxia. Furthermore, qPCR analysis revealed that HIF-2 α target transcripts significantly decreased in the presence of ISGylation in hypoxia. Interestingly, the ISGylation system has been identified to contain HREs. qPCR analysis suggests that ISGylation expression is only transiently induced in hypoxia. Therefore, ISGylation likely participates in a negative feedback loop of HIF-1 α and HIF-2 α -mediated gene expression in hypoxia. This work highlights ISG15 as a regulatory modifier of HIF-2 α transcriptional activity in cancer cells, and provides insight into a potentially new avenue for therapeutic treatment.

Funding: Canadian Cancer Society

19) *Increasing the Magnitude of Tumour-Specific T Cell Responses by Spreading a Vaccine Dose Across Multiple Injection Sites*

Robert Mould¹, Amanda Au Yeung¹, Geoffrey Wood¹, Sarah K. Wootton¹, Leonardo Susta¹, James J. Petrik², Anthony Mustaers² and Byram W. Bridle^{1*}

Departments of ¹Pathobiology, ²Biomedical Science, OVC, University of Guelph

Vaccination is a common immunotherapy to generate potent humoral and cell-mediated immunity. Cancer patients require rapid induction of robust tumour-specific effector T-cell responses to halt neoplastic growth and hopefully eliminate malignant cells. Immunological tolerance imposes strong selection pressures against autoreactive T cells. Therefore, T cells that can respond to vaccines targeting self-tumour-associated antigens (TAA) are usually at low frequency. In clinical settings, vaccines are often administered as single bolus injections. We reasoned that administration of a cancer vaccine at multiple injection sites would increase the amount of secondary lymphoid tissue involved in antigen presentation, thereby maximizing the engagement of low frequency TAA-specific T cells. To test this, C57BL/6 mice were vaccinated with a replication-deficient human serotype 5 adenovirus expressing the TAA dopachrome tautomerase (Ad5-DCT). 1x10⁸ plaque-forming units of Ad5-DCT was injected intramuscularly into one (hamstring or triceps brachii), two (both hamstrings or both triceps brachii) or four (both hamstrings and both triceps brachii) sites. The magnitude of the CD8⁺ T cell response against the immunodominant epitope of DCT (DCT180-188) increased with the number of injection sites. Enhanced responses associated with multiple vaccination sites were evident at the peak of the primary response as well as post-contraction. These results suggest it may be beneficial to

administer cancer vaccines to patients at more than one site. Whether this has implications for vaccines beyond those targeting self-antigens remains to be tested.

Funding: Terry Fox Research Institute, Art Rouse Cancer Biology Graduate Stipend

20) *Development of drug resistance in colorectal cancer cells is associated with changes in the expression of chemokines*

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One of the recently recognized hallmarks of cancer highlights evasion from immune destruction as a potential route that cancer cells take in order to survive in the blood circulation and extravasate into the secondary tissue vasculature. In addition, cancer cells often exploit certain pathways normally utilized by immune system cells to facilitate their route to form metastases. We are focusing on the roles of chemokines and their receptors in colorectal cancer (CRC) metastasis. We have developed a drug-resistant counterpart to the HT-29 CRC cell line, HT-29S, that is resistant to the SN-38 active metabolite of the chemotherapeutic drug irinotecan, one of the agents used in CRC treatment regimens. Eleven chemokine-receptor combinations were examined in these paired CRC cell lines and the mRNA expression was carried out using end-point PCR. Out of the 22 targets screened, six had a potential shift in the expression between the parental and drug-resistant cells. Expression of CCL2 and CXCL12 was up-regulated whereas that of CCL15, CXCL8, CCL20 and CXCR4 was decreased in the drug-resistant cells. The alterations in chemokine/receptor expression were confirmed using a quantitative approach (qPCR). These changes suggest that as CRC cells become drug-resistant during systemic chemotherapy, there are alterations in pathways normally utilized by the immune system, which facilitate the ability of the cancer cells to traverse the vasculature, extravasate and seed metastases in other organs such as the liver. Further studies will allow for a better understanding of the metastatic process.

Funding: NSERC

21) *The impact of cancer and chemotherapy on whole blood niacin status in dogs*

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Niacin deficiency has been observed in human cancer patients, and is associated with genomic instability. Based on previous non-human studies, normalizing niacin levels with supplementation restores the ability of healthy cells to signal DNA repair upon exposure to alkylating agents, which may reduce the incidence of secondary malignancies and acute side effects of chemotherapy. This observational study aims to assess niacin status in healthy dogs (n=18), as well as dogs suffering from lymphoma (LPA; n=28), mast cell tumour (MCT; n=25), or osteosarcoma (OSA; n=19), at presentation and throughout their treatment. It provides

foundation for future intervention studies to incorporate niacin supplementation into anti-cancer regimens. This study assesses the value of whole blood NAD/NADP, hematocrit-adjusted NAD/NADP, and Niacin Index as niacin status parameters during canine cancer treatment. Client-owned dogs were screened for confounding diseases or conditions. Blood was collected at first presentation for all three cancer types and at relevant follow-up time points for LPA and OSA. Enzyme-driven colorimetric assays quantified NAD(H) and NADP(H) pools. MCT was not statistically different from the healthy group in any parameter ($P>0.05$). LPA, OSA and Combined cancer groups had a significantly reduced niacin status at baseline compared to healthy ($P<0.0001$), and their niacin status improved over the course of treatment ($P<0.0001$). Dogs presenting with LPA and OSA, but not MCT, have a significantly reduced niacin status when compared with healthy dogs, and niacin status of patients continuing treatment recovered by the first follow-up. Canine cancer patients may benefit from niacin supplementation as indicated by past non-human experiments.

Funding: OVC Pet Trust Fund, AAVN-Waltham Research Grant

22) *Lack of Autophagy Alters Mitochondrial Phenotype Resulting in Altered Chemosensitivity of Acute Myeloid Leukemia*

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Autophagy is a catabolic process by which damaged, defective, or long-lived cellular components are engulfed by an autophagosome and subjected to lysosomal degradation. In addition to acting as a homeostatic mechanism, autophagy can be initiated in response to cellular stress as an adaptive survival mechanism. Lack of autophagy results in an accumulation of damaged or dysregulated organelles, a common feature of mitochondria in AML. Here, we sought to better understand the link between genes involved in mitochondrial maintenance and sensitivity to chemotherapeutic agents that target mitochondria. Knockdown of autophagy/mitophagy genes NIX (gene: BNIP3L) and p62 (gene: SQSTM1) produced cells with increased reactive oxygen species (ROS) and altered mitochondrial membrane potentials that were more sensitive to a variety of mitochondrial targeting chemotherapeutic agents. Our results suggest a link between autophagy proteins and AML drug sensitivity, which could have important future prognostic implications.

Funding: Leukemia & Lymphoma Society of Canada, Canadian Hematology Society

23) *An Anti-Leukemic Flavonoid Targeting the Estrogen Receptor β*

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Acute myeloid leukemia (AML) is an aggressive hematological malignancy resulting from the accumulation of immature myeloid cells in the peripheral blood and bone marrow that develop from leukemia stem cells (LSCs). To improve patient prognosis, new LSC-targeting therapies

are needed. To address this need, we created a unique library consisting of 288 nutraceuticals which was screened against TEX cells, a surrogate LSC line. Among these compounds, the flavonoid diosmetin targeted TEX cells most potently (EC₅₀: 6.1 ± 0.5 μM, p<0.001) without affecting non-LSC K562 cells, suggesting that diosmetin may selectively target leukemia stem cells. Furthermore, diosmetin (10 μM) reduced clonogenic growth in primary AML patient samples (n=6) but not in CD34+ normal hematopoietic cells (n=3). Finally, compared to vehicle control, diosmetin delayed tumor growth in AML mouse xenografts (p<0.01). To identify diosmetin's molecular target we utilized online bioinformatics tools (e.g., protein data base, PoSSuM and DAVID tool), which identified the estrogen receptor (ER) as diosmetin's potential target. To confirm this, we measured ER α and ER β levels in various cell lines and patient derived AML cells. Interestingly, diosmetin sensitive cell lines (TEX, LP1) and patient samples displayed significantly elevated ER β levels compared to those insensitive (K562, DU145). This pattern was not observed for ER α. Finally, shRNA knockdown of ER β caused diosmetin insensitivity while ER reporter assays demonstrated that diosmetin acts as an agonist in ER β but not ER α reporter cells. Together, these studies highlight diosmetin binding to ER β as a potential novel strategy for the treatment of AML.

Funding: Stem Cell Network, University of Waterloo

24) *Insulin-Like Growth Factor 1 Receptor Expression in Canine Appendicular Osteosarcoma using Tissue Microarray and Automated Image Analysis*

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Currently, the number of relevant biomarkers for canine appendicular osteosarcoma (OSA) is limited. Receptor tyrosine kinases (RTKs) are recognized as valuable biomarkers as well as potential therapeutic targets in a variety of human and canine cancers. A canine appendicular OSA tissue microarray (TMA) containing 175 clinically annotated cases was constructed to aid with biomarker discovery. Immunohistochemistry for the RTK insulin-like growth factor 1 receptor (IGF-1R) was performed on the OSA TMA and slides were imaged by the Digital Histology Shared Resource at Vanderbilt University Medical Center. An image analysis algorithm was designed using Leica SlidePath software via Vanderbilt's Digital Image Hub. This preliminary algorithm was used to analyze a subset of 30 cases from the OSA TMA. The median survival time (MST) was 210 days (range 18-1244). Neoplastic cells displayed weak, moderate, and strong cytoplasmic labeling. The total number of strongly labeled cells, the overall proportion of strongly labeled cells, and the number of strongly labeled cells per mm², were all significantly correlated (p < 0.05) with decreased survival time. Kaplan Meier survival functions for high (n=13, MST=140) and low (n=17, MST=324) IGF-1R expression were compared using the log-rank test. High IGF-1R expression was significantly correlated with decreased survival time (Chisq: 6.1, 1 df, p < 0.05). This preliminary data suggests that IGF-1R is a candidate prognostic biomarker for canine appendicular OSA and highlights the utility of both TMA and image analysis technology for biomarker discovery in veterinary oncology.

Funding: OVC Pet Trust Fund

25) *The ICCI Companion Animal Tumour Sample Bank: Canada's Only Veterinary Oncology Biobank*

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The Companion Animal Tumour Sample Bank (CATSB) continues to successfully facilitate basic and translational veterinary oncology research. Currently, CATSB has over 700 cases banked and has contributed samples to 15 research projects. Located in the OVC HSC Mona Campbell Centre for Animal Cancer, the CATSB is the only veterinary oncology tissue bank in Canada and was recently registered with the Canadian Tissue Repository Network. Sample types collected and stored at ultracold temperature are: serum, plasma, buffy coat, urine, and tissue. Tissue samples, both tumour and matched normal, are collected immediately following surgical excision and are available as flash frozen, in RNAlater, and in CryoMatrix. Tumour tissue is also formalin fixed, paraffin embedded, sectioned, and H&E stained for quality control analysis by a pathologist. There are currently 9 primary cell lines from canine and feline tumours available, with more in development. The three most prevalent canine tumour types are soft tissue sarcoma, osteosarcoma, and mast cell tumours, but a variety of other neoplasms have also been banked. Researchers access samples by filling out a short application form. Prospective sampling can also be tailored to suit the needs of researchers. A cost-recovery fee (which is subsidized for University of Guelph researchers) is assessed to enable the CATSB to continue its mission. In addition to samples, researchers can receive patient signalment, histopathology, and follow-up data. Via the CATSB, the ICCI strives to facilitate research to improve the lives of companion animals with cancer, with the potential to contribute to comparative human cancer research.

Funding: OVC Pet Trust Fund

26) *Inhibiting the PI3K/mTOR signaling pathway in canine melanoma cell lines*

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Melanoma accounts for up to 7% of malignant tumors in canines. It is a very aggressive disease, manifesting with a high degree of local invasiveness, and high metastatic potential. Local tumour control may be achieved through surgery and/or radiation therapy. There has been limited success in achieving systemic control with cytotoxic chemotherapeutics, such as carboplatin. As a result, canine melanoma is considered to be a highly chemotherapy resistant tumour and most dogs succumb to metastatic disease within approximately one year. Therefore, it is crucial to continue to explore novel therapeutic strategies for canine melanoma. One promising area may be through the targeting of aberrant cellular signaling pathways in this disease. Several kinases in the PI3K/mTOR pathway have been shown to be upregulated in canine melanoma, aiding tumour cell proliferation, growth, and chemoresistance. The objectives of this study were to determine the efficacy of inhibiting PI3K and mTOR alone and in combination on canine melanoma cell proliferation and response to chemotherapy treatment. Five canine melanoma cell

lines were treated with PI3K inhibitor LY294002, or PI3K/mTOR dual inhibitor GSK2126458, alone and combined with carboplatin. Cell proliferation was assessed using the crystal violet assay and IC50 values were determined. GSK2126458 was more potent than LY294002 in decreasing cell proliferation. Western blot comparison analysis of signaling pathway components PI3K, Akt, mTOR, and p70S6K is ongoing. Future studies will evaluate single agent mTOR inhibition, investigate further mechanistic effects such as apoptosis and clonogenic survival, and assess the impact of drug scheduling on outcome.

Funding: OVC Pet Trust Fund, OVC Scholarship

27) Sensitization of Leukemic Cells to Oncolytic Viruses Using Epigenetic Modifiers
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Acute lymphoid leukemias are blood-derived cancers usually originating from B- (B-ALL) or T cells (T-ALL). Treatments for B-ALL in children have high survival rates but compromise quality of life. T-ALL is much less treatable, and adults with either disease have poor prognoses. Alternatives include oncolytic viruses (OVs) and epigenetic modifiers (EMs) that preferentially kill tumour cells. Others have used EMs to potentiate OVs in solid tumours. We hypothesized that EMs could similarly potentiate OVs in leukemias. We used L1210 and EL4 cells as models of B- and T-ALL, respectively. Cells were treated with EMs and OVs as monotherapies and in combinations. Several promising combinations were identified. Furthermore, cells that survived treatment with high doses of EMs or OVs as monotherapies were passaged several times and then tested for resistance to other treatments. Interestingly, cells that survived treatment with EMs were more sensitive to OV-mediated killing than the parental cell lines. Notably, this EM-mediated sensitization to OVs was most dramatic in T-ALL cells. Exposure to decitabine was particularly effective at increasing sensitivity to OVs. Mice were challenged with L1210 cells that had survived treatment with decitabine or parental cells. This was followed by administration of oncolytic maraba virus. Control groups that did not receive viruses reached endpoint at the same time. Among groups treated with maraba virus, those challenged with decitabine-selected L1210 cells survived significantly longer. EMs, especially decitabine, appear effective in potentiating OV-mediated treatment of leukemias, especially T-ALL.

Funding: Cancer Research Society, OVC Scholarship

28) Attenuation of Wnt signaling by Nkd1 in cases of abnormally elevated signaling activity
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Wnt signaling pathways play a critical role in controlling development and regulating stem cell populations in adult organisms. Wnt signaling events in embryogenesis includes establishment of the dorsal organizer, ventrolateral mesoderm patterning and formation of the anterior-posterior

neuroectoderm. Nkd1, a feedback regulator of Wnt signaling, is hypothesized to regulate signal intensity when signaling levels breach an unidentified homeostatic threshold. Using CRISPR/Cas9 genome editing Nkd1 function was investigated in early zebrafish embryogenesis. nkd1 knockdowns were hypersensitive and phenotypically dorsalized and ventralized in cases of ectopic pathway overactivation. These findings reinforce the idea that Nkd1 functions as a passive antagonist of Wnt signaling and provide a basis for further investigation into Nkd1 activity.

Funding: NSERC

29) Investigation of Calcitriol's Effects on Cell Viability in Canine Osteosarcoma Cell Lines
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Background – Canine osteosarcoma is a common, malignant bone tumor that has a high likelihood of metastasis to other tissues and organs. Current chemotherapy, when used to treat established metastatic osteosarcoma, has marginal benefit. Calcitriol, the most active form of vitamin D, has shown significant anti-cancer effects in human cell lines. It may have activity alone or could, in combination, potentiate the effects of chemotherapy.

Objective – To investigate the dose/response effects of calcitriol on cellular viability of two, well-characterized, canine osteosarcoma cell lines. Methods – D-17 and Duke cell lines were treated with graded concentrations of calcitriol, from 1 nM to 1 μM at 1000 and 2000 cells/ well of a 96-well plate. For the D-17 cell line, two trials with end-points of 72 h and 96 h were completed. Thus far, one trial has been completed on the Duke cell line using the same end-points. Cellular viability was assessed using the resazurin assay. Data were analyzed using one-way ANOVA (normally distributed) and Kruskal-Wallis (not normally distributed). Results – Cell viability was reduced in Duke cells after 72h by a maximum of 31% (p=0.0002). In contrast, a reduction in D-17 cell viability was only observed in the first trial of the 96-hour treatment at 2000 cells/well density by a maximum of 10.2% (p=0.0305). Conclusions – Based on these results, calcitriol's effects on cell viability within each cell line was variable depending on the concentration used and the treatment duration. Combination treatment with a chemotherapy agent might evoke a more substantial reduction in cell viability.

Funding: OVC Pet Trust Fund

30) Targeting the PI3K/mTOR Pathway in Sensitizing Canine Osteosarcoma Cells to Chemotherapy

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Osteosarcoma (OS) is the most common primary bone cancer found in both humans and dogs. While considered an orphan disease in humans, OS is highly prevalent in dogs, accounting for over 10,000 cases per year. OS has a very poor prognosis, with survival rates of 20-30% in

humans and 10-15% in dogs. Minimal improvement of survival rate has been achieved over the past 3 decades making this disease of high priority for the development of new treatment strategies. Recently, the PI3K/mTOR pathway has been indicated as a vulnerable target during OS treatment. Playing a role in cell cycle regulation, this pathway has been shown to be overactive in many forms of cancer. To date, many drugs have been developed targeting substrates within the pathway. In this study, we assess the effects of single and dual inhibitors of this pathway on canine OS cellular viability *in vitro* when combined with standard chemo treatment. Results demonstrate that dual inhibition of both PI3K and mTOR lead to a more robust treatment response to chemotherapeutic drugs; highlighting the benefits of this approach in future treatment strategies in canine OS.

Funding: OVC Pet Trust Fund

31) *An Integrated Approach for Characterizing the Transcriptomic and Proteomic Landscape of Neuronal Adaptor Protein ShcD*

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The majority of cellular processes are modulated by protein-protein interactions that serve as the basis for intracellular signaling cascades. Perturbations in these cascades can lead to abnormal signaling events ultimately resulting in diseases like cancer. Specialized phosphotyrosine circuitry translates extracellular signals into cellular responses through altered phosphorylation states of tyrosine residues using receptor tyrosine kinases, phosphatases, and modular interaction domains such as Src homology 2 (SH2) and phosphotyrosine binding (PTB) domains. Prototypical adaptors such as the Shc (Src homology and collagen) family of four evolutionarily related proteins contain both domains enabling them to act as cytosolic sensors and respond to altered tyrosine phosphorylation states upon receptor engagement. The most recently isolated member ShcD, however, appears to challenge the canonical Shc signaling model. Through its PTB domain, ShcD binds to and facilitates ligand-independent autophosphorylation of EGFR. Interestingly, ShcD is also transcriptionally upregulated in gliomas, which are highly associated with misregulated EGFR signaling. Furthermore, ShcD phosphorylation has been shown to repress Erk activation downstream of the neurotrophic receptor TrkB. It is hypothesized that phosphorylated ShcD binds and sequesters another adaptor protein, Grb2, away from meaningful signaling to Erk and this interaction is regulated by a phosphatase recruited by the ShcD SH2 domain. Mass spectrometric analysis of phosphorylated ShcD has identified the phosphatase Shp2 as a binding partner. This project, therefore, seeks to investigate further the mechanisms through which ShcD impinges on signal transduction using a multi-pronged approach combining Bioinformatics tools with *in vitro* and *in vivo* validation models.

Funding: NSERC

32) *Inhibition of eIF4E2 in colorectal cancer leads to a reduction in autonomous proliferation and increased migration under hypoxia*

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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 eIF4E2, initiating the selective translation of hypoxia-response mRNAs. Cancer cells exploit this pathway for their proliferation, migration and survival. Here we show that human colorectal cells harbouring shRNA against eIF4E2, are impaired in their ability to autonomously proliferate but gain a migratory advantage under hypoxia and maintain cell-cell contact in a spheroid formation assay. By characterizing the role of eIF4E2 in colorectal cancer, this project will not only enhance our understanding of tumor biology but may also offer an interesting avenue for future therapeutic interventions. Which will target hypoxic areas, such as the core of tumors and leave the standard protein synthesis machinery unharmed.

Funding: Cancer Research Society, CFI

33) Examination of the effects of hyperthermia and HSP70 expression on phosphorylation and regulation of FoxO3a during heat stress

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Cells respond to stressful stimuli by activating an evolutionarily conserved ‘heat shock response’ that results in the increased production of heat shock proteins. These proteins, particularly HSP70, act as part of a defense mechanism to limit the extent of protein damage and thereby allows cells to survive proteotoxic stresses. FoxO3a is a transcription factor that functions to activate transcription of pro-apoptotic genes facilitating cell death. Under physiological conditions FoxO3a associates with 14-3-3 proteins in the cytoplasm following phosphorylation by Akt at T32, S253, and S315 sites. Cytoplasmic localized FoxO3a is ubiquitinated and degraded by the proteasome. In the absence of growth factors FoxO3a becomes dephosphorylated, loses its association with 14-3-3 and translocates to the nucleus resulting in transcriptional activation. In this study we examined the potential role of hyperthermia in the activation of FoxO3a and the influence of HSP70 on FoxO3a function. Using a human T cell line with tetracycline-inducible expression of HSP70 we demonstrated that hyperthermia causes nuclear translocation of FoxO3a and that this is inhibited in cells expressing HSP70. S253 phosphorylated FoxO3a also translocates to the nucleus suggesting that loss of Akt-mediated FoxO3a phosphorylation is not responsible for the heat-induced nuclear translocation. Hyperthermia also led to an increased abundance of FoxO3a, however when HSP70 was present FoxO3a was rapidly depleted. Together these results provide evidence that stress-induced apoptosis is mediated by the activation of FoxO3a and that HSP70 prevents stress-induced apoptosis by preventing the nuclear translocation of FoxO3a and accelerating its destruction.

Funding: NSERC

34) *Characterizing Wnt signaling activation and activity as regulated by Nkd1 in mammalian cell lines IEC-18 and HEK293*

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The Wnt signaling pathway controls a multitude of developmental and regulative processes including patterning, cell proliferation, differentiation, and migration. Normally, the Wnt signaling pathway is tightly regulated, and mutations resulting in consecutive activation of this pathway are a major driving force behind the initiation of many cancers. Nkd1 is an intrinsic negative feedback regulator of the Wnt pathway, though many aspects of its function and activity are not well understood. Here I explore how activation of the Wnt pathway in two mammalian cell lines, HEK293 and IEC-18, effects Nkd1 function. Understanding the function of Wnt negative feedback regulators will provide a perspective on why they are unable to restrict oncogenic development, and potentially assist with the development of future cancer therapeutics.

Funding: NSERC, OGS

35) *siRNA-mediated knockdown of PRKAR1A in canine osteosarcoma cells increases autophagy response to chemotherapy*

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Canine osteosarcoma (OSA) is a highly malignant form of bone cancer in dogs, accounting for up to 85% of malignancies originating in bone. Without treatment, the mean survival time is less than 3 months. Over 90% of OSA patients develop pulmonary metastases within 1 year of diagnosis despite standard of care treatment, which consists of surgical amputation followed by adjuvant chemotherapy. The high mortality rate of OSA could be due to the ability of OSA cells to resist chemotherapy-induced cell death by entering autophagy. Autophagy, or “self-eating”, is an evolutionarily conserved intracellular catabolic process which plays an important role in adaptation to environmental changes and maintenance of cellular homeostasis. Cancer cells have been shown to enter autophagy and become resistant to chemotherapy; however, the role of autophagy in OSA is currently unknown. Human and canine OSA have numerous similarities including variation in the expression of a gene called PRKAR1A. Patients with low PRKAR1A-expressing OSA have longer post-chemotherapy survival times than high PRKAR1A-expressing OSA in both species. The conversion of LC3-I to LC3-II protein is a maker of autophagy. LC3 processing was analyzed by Western blot. Compared to the negative control, PRKAR1A knockdown canine OSA cells showed an increased accumulation of LC3-I when treated with doxorubicin. Our findings suggest that the expression of PRKAR1A may be involved in the regulation of autophagy and that targeting PRKAR1A could potentially increase chemosensitivity in the treatment of canine OSA.

Funding: OVC Pet Trust Fund