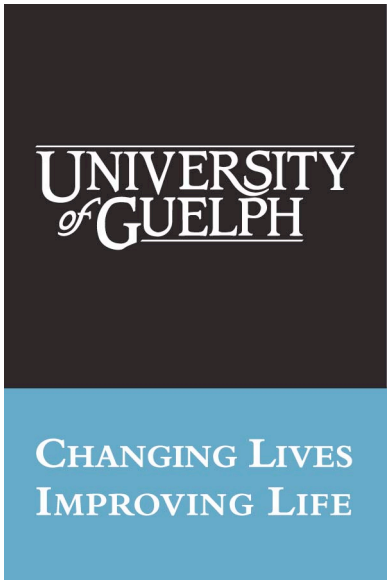
OVC | Institute for Comparative Cancer Investigation | University of Guelph

7th Annual Cancer Research Symposium Program

Thursday, May 15, 2014
Room 1714 LLC, Ontario Veterinary College
University of Guelph



Introductory Remarks

Welcome to the 7th Annual Guelph ICCI Cancer Symposium. This meeting is intended to bring together individuals interested in the study of any aspect of cancer in any species, from the most basic elements, to clinical therapies and on to social, emotional and ethical aspects of this often-devastating disease. Again this year we are highlighting local, national and international researchers.

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

Co-Organizers
Tony Mutsaers and Brenda Coomber
Clinical Studies and Biomedical Sciences, University of Guelph

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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: The Canadian Cancer Society; The Cancer Research Society; Leukemia & Lymphoma Society of Canada; US Army Department of Defense Breast Cancer Research Program; Canadian BC Foundation (Ontario Chapter), Ontario Institute for Cancer Research; Canadian BC Research Alliance; Terry-Fox Foundation; CIHR; NSERC; CFI; Ontario MRI; OGS; AAVN-Waltham; Royal Canin; University of Waterloo; OVC Pet Trust; Arthur Rouse Graduate Stipend; College of Biological Science, Department of Pathobiology and Office of the Dean, Ontario Veterinary College, University of Guelph.

ICCI 7th Annual Cancer Research Symposium
Thursday May 15, 2013

Morning Session: Room 1714, OVC LLC

9:00 - 9:15 Welcome and Introductory Remarks

9:15 - 9:45
Dr. Jim Uniacke, Molecular & Cellular Biology, University of Guelph
Cancer cells require eIF4E2-directed hypoxic protein synthesis en route to tumor progression

9:45 - 10:00 short talk from submitted abstracts
Robert Jones, Biomedical Sciences, University of Guelph
The Role of Rb and p53 in Breast Cancer: Insights into Cellular Origins and Identification of Novel Therapies

10:00 - 10:30 ***Coffee Break*** **Room 1707 B & C, OVC LLC**

10:30 - 11:00
Dr. Krista Power, Guelph Food Research Centre, Agriculture and Agri-food Canada, Guelph
The role of dietary flaxseed in modulating colon health and inflammation in mice

11:00 - 11:15 short talk from submitted abstracts
Leonard Angka, School of Pharmacy, University of Waterloo
Glucopsychosine, a lipid derived from bovine milk, increases cytosolic calcium to induce calpain mediated apoptosis of acute myeloid leukemia cells

11:15 - 12:00 Guest Speaker
Dr. John Bartlett, Director of Transformative Pathology, Ontario Institute for Cancer Research, Toronto
Predicting anthracycline benefit in early breast cancer

12:00- 1:30 **Room 1707 B & C, OVC LLC**
Poster Session and Lunch (provided)
poster presenters please attend your posters between 12:30 and 1:30

Afternoon Session: Room 1714, OVC LLC

1:30 - 2:00
Dr. April Khademi, Biomedical Engineering, School of Engineering, University of Guelph
Image analysis solutions for the automatic analysis of pathology specimens

2:00 - 2:15 short talk from submitted abstracts
Christian Ternamian, Pathobiology, University of Guelph
Oncolytic Rhabdoviruses in Combination with Histone Deacetylase Inhibition Synergistically Kill Murine B Lymphoblastic Leukemia Cells

2:15 - 2:30 short talk from submitted abstracts
Jaijie Liu, Human Health & Nutritional Science, University of Guelph
Plant- and marine-derived n-3 polyunsaturated fatty acids reduce mammary gland tumor development in MMTV-neu-YD5 mice

2:30 - 2:45 short talk from submitted abstracts
Nicole Weidner, Clinical Studies, University of Guelph
Dietary vitamin D intake and vitamin D status in canine cancer patients

2:45- 3:15
Dr. Valerie Poirier, OVC HSC Animal Cancer Centre
Radiation, new with old

3:20-3:30 Break

3:30 - 4:30
Keynote Speaker
Dr. Deborah W. Knapp, Purdue Comparative Oncology Program, School of Veterinary Medicine, Purdue University
Urinary Bladder Cancer – Applying Comparative Oncology Research to Transform the Outlook for Humans and Dogs

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

KEYNOTE PRESENTATION

3:30 OVC LLC Room 1714

Dr. Deborah W. Knapp

DVM, MS, Dipl. ACVIM
Dolores L. McCall Professor of Comparative Oncology, Director, Purdue Comparative Oncology Program, Department of Veterinary Clinical Sciences, School of Veterinary Medicine
Purdue University

Urinary Bladder Cancer – Applying Comparative Oncology Research to Transform the Outlook for Humans and Dogs

Each year, approximately 70,000 people in Canada and the United States die from urinary bladder cancer. Most deaths are due to high-grade invasive urothelial carcinoma, also referred to as invasive transitional cell carcinoma (TCC). Pet dogs also "spontaneously" develop invasive TCC, with >20,000 dogs developing this cancer yearly in Canada and the United States. Although TCC is not usually curable in dogs, it is considered a highly "treatable" condition with >75% of dogs enjoying many months to a year or more of good quality life after diagnosis. Naturally-occurring invasive TCC in dogs is exquisitely similar to invasive bladder cancer in humans, and serves as a highly relevant model of the human condition. Research is ongoing in dogs to gain a better understanding of risk factors and to develop strategies for TCC prevention, to find the cancer earlier when it may respond better to treatment, to predict how the cancer will behave in individual patients, and to treat it more effectively. The goal is to improve the outlook for humans and dogs that face this cancer.

Dr. Knapp received her B.S. degree from North Carolina State University in 1980, her D.V.M. from Auburn University in 1983, and M.S. degree from Purdue University in 1988. She also completed an oncology residency and post doctoral experience in cancer pharmacology at Purdue University before joining the faculty there in 1990. Her faculty position involves didactic and clinical teaching, clinical and bench research, and clinical and other service responsibilities. Dr. Knapp's research is strongly focused in the area of invasive urinary bladder cancer in which advances in dogs with naturally-occurring bladder cancer are being used to improve the outlook for pet dogs with this cancer and to translate important findings into studies in humans. She is senior author on more than 35 peer-reviewed publications in the urinary bladder cancer field, plus numerous collaborative papers and book chapters. Dr. Knapp is co-director of the Bladder Cancer Focus Group, a joint interdisciplinary research group between the NCI designated Purdue University Center for Cancer Research (PUCCR) and the Indiana University Simon Cancer Center. She serves on the Executive Committee of the PUCCR and is the Co-Program Leader for the Medicinal Chemistry Program within the Center. She also serves on the Advisory Committee for the Cancer Prevention Program within Purdue's Oncological Sciences Center, and the Cancer Prevention Internship Program for undergraduate and graduate students at Purdue University.

Past ICCI Symposium Arthur Willis Distinguished Speakers

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

GUEST SPEAKER

11:15 OVC LLC Room 1714

Dr. John Bartlett, PhD

Director of Transformative Pathology, Ontario Institute for Cancer Research, Toronto

Predicting anthracycline benefit in early breast cancer

Following training as a Biochemist and Reproductive Endocrinologist, Professor Bartlett switched to translational cancer research in 1990. During his time at Glasgow and Edinburgh Universities (1993-2011) John built an international reputation as a translational research scientist which lead to the award of a Fellowship of the Royal College of Pathologists and a Personal Chair at Edinburgh University. During this time he also developed the UK NEQAS scheme for HER2 ISH testing and was responsible for a number of key publications in the field. Having recently moved to Toronto, Professor Bartlett has now published over 150 research articles and is co-author on the UK guidelines for HER2 testing.

ORAL PRESENTATION ABSTRACTS: MORNING SESSION (*Study Leader)

Invited Local Speaker

Jim Uniacke, Department of Molecular & Cellular Biology, University of Guelph*
Cancer cells require eIF4E2-directed hypoxic protein synthesis en route to tumor progression.

Over one hundred cancer types have been identified in different organs. Research in the oncology field has demonstrated that cancer is a multifactorial disease and the paths taken by cells en route to malignancy are highly variable. Yet, solid tumors share characteristics regardless of their tissue of origin or genetic makeup. These characteristics are collectively referred to as the "tumor microenvironment" and include factors such as hypoxia (low oxygen tension), nutrient deprivation, irregular vascularization, and extracellular acidosis. Cancer cells must cope with hypoxia within tumors as oxygen diffuses only through roughly a dozen cellular layers or 150 um mfrom a blood vessel. This presents a challenge to the classical translation apparatus as hypoxia is a potent inhibitor of cap-dependent translation. We have shown that hypoxic cells switch from eukaryotic initiation factor 4E (eIF4E) to eIF4E2 cap-dependent translation to synthesize many of their proteins. Here, we show that genetically distinct human cancer cells exploit eIF4E2-directed protein synthesis to form cellular masses larger than approximately 0.15 mm, the diffusion limit of oxygen. Cancer cells depleted of eIF4E2 are indistinguishable from control cells under normoxic conditions, but are unable to survive and proliferate in low oxygen conditions. Activation of eIF4E2-directed translation is essential for cancer cells to form a hypoxic tumor core in in vitro spheroids and to form detectable tumors in in vivo xenograft assays. In contrast, the eIF4E-directed protein synthesis pathway alone cannot sustain cellular adaptation to hypoxia in vitro or confer tumorigenic potential in xenograft assays. These data demonstrate that the phenotypic expression of the cancer genome requires translation by the eIF4E2-directed hypoxic protein synthesis machinery.

Short Talk from Submitted Abstracts

Robert Jones[1,2], Zhe Jiang[1], Hui Qin Li[1], Jeff C. Liu[1], Tyler Robinson[1], Philip Cheung[1], Aaron Schimmer[3] and Eldad Zacksenhaus[1]; [1]Division of Cellular & Molecular Biology, Toronto General Research Institute, University Health Network, Toronto; [2]Department of Biomedical Sciences, University of Guelph; [3]Ontario Cancer Institute, University of Toronto*
The Role of Rb and p53 in Breast Cancer: Insights into Cellular Origins and Identification of Novel Therapies

Triple-negative breast cancer (TNBC) is an aggressive form of the disease that is defined by the absence of hormone receptors (estrogen and progesterone) and human epidermal growth factor receptor 2 (HER2) expression. While effective therapeutic agents targeting these receptors are currently in use (eg. Tamoxifen, Herceptin), conventional chemotherapy remains the only available treatment for TNBC. Hence, there is great interest in understanding the biology of tumors with triple-negative status and the identification of more specific and less toxic therapies. At the genetic level, two key tumor suppressor genes, RB1 (retinoblastoma protein 1) and p53,

are frequently lost in TNBC. To directly test whether RB1 has a causative role in breast cancer, we deleted mouse Rb in the stem/bipotent progenitor cell compartment of the mammary gland. This led to the formation of diverse mammary tumors including TNBCs, a subset of which contained oncogenic mutations in p53. These tumors displayed features of basal-like breast cancer or epithelial to mesenchymal transition (EMT), both of which are correlated with triple-negative status in human breast cancer. Accordingly, combined inactivation of Rb and p53 accelerated tumor formation and induced triple-negative EMT-type tumors. Interestingly, deletion of Rb in alveolar progenitor cells failed to induce tumor formation while combined deletion of Rb and p53 throughout the mammary epithelial hierarchy was tumorigenic. Integration of copy number, gene expression and FDA-approved drug screen data identifed the antibiotic Tigecycline as well as Met receptor and MMP13 inhibitors as potential therapies for Rb/p53-deficient TNBC. Together, this work demonstrates a causal role for Rb in breast cancer and the influence of cell type on transformation-susceptibility. These results also illustrate the importance of cooperating oncogenic events in dictating tumor subtype and the use of genetically engineered mouse models to identify novel therapies for breast cancer treatment.

Invited Local Speaker

Krista Power, Guelph Food Research Centre, Agriculture and Agri-food Canada, Guelph*
The role of dietary flaxseed in modulating colon health and inflammation in mice

Colonic inflammation plays an important role in the development and progression of colitis-assocaited colon cancer (CAC). Dietary bioactives (e.g. prebiotics and n3-polyunsaturated fatty acids) modulate colitis and CAC through their effects on gut microbial activity and community structure, gut barrier intergity, and the mucosal immune response. Recently, we demonstrated that although rich in soluble fibre and α-linoleneic acid, consumption of 10% flaxseed (FS) diet supplemented to AIN-93G basal diet (BD), worsened the severity of dextran sodium sulfate (DSS)-induced colitis in C57Bl/6 mice, as indicated by increased colonic damage, inflammatory cytokines, and immune cell infiltration. In healthy mice, FS consumption altered the colonic expression of 309 genes compared to BD controls (169 up and 140 down regulated) using Affymetrix MoGene ST 1.0 arrays. Mapping differentially expressed genes into the Reactome functional interaction network revealed that several biological networks related to the cell cycle (e.g. PCNA, Cyclin A2, and Cyclin B2) were affected by FS diet, the majority of which were down-regulated. To help elucidate the mechanisms of action, FS bioactives (oil, mucilage, and protein (FP)) were extracted and examined for their role in modulating colitis and colon health. Similar to the effects of whole FS, mice fed 20% FP aggravated DSS-induced colitis severity as indicated by increased colonic histological damage and inflammation compared to BD controls. In the healthy colon, FP adversely affected the gut barrier (reduced proliferation and mucus production), modulated microbial community structure (eg. increased Bacteriodetes and reduced Firmicutes) and activity (e.g. increased protein fermentation products), and reduced expression of genes involved in maintaining gut barrier integrity (TFF3 and Relmβ), adherens and tight junctions (JAM-A and ZO-1), and Toll-like receptors (TLR4 and TLR9). In conclusion, consumption of dietary FS and its protein fraction, modulates biomarkers of colon health which adversely influences colonic inflammation and potentially CAC.

Short Talk from Submitted Abstracts

Angka L, Lee EA, Rota SG, Hanlon T, Sukhai M, Minden M, McMillan EM, Quadrilatero J, Spagnuolo PA; School of Pharmacy, University of Waterloo*

Glucopsychosine, a lipid derived from bovine milk, increases cytosolic calcium to induce calpain mediated apoptosis of acute myeloid leukemia cells

Acute myeloid leukemia (AML) is a devastating disease with only 5-35% of adult patients surviving past 2 years. To identify potential novel AML therapeutics, we created and screened a unique library consisting of food-derived bioactive compounds with previously unrecognized anti-cancer activity. Here, we identified glucopsychosine (GLU), a lipid derived from bovine milk, as a novel anti-AML agent. GLU induced death of AML cell lines (IC50: 5-10μM) and primary AML patient samples but had no effect on cells obtained from normal marrow. Given the in vitro effects, GLU was next evaluated in leukemia mouse models. GLU decreased tumor weight up to 4-fold compared to control without evidence of weight loss or changes in serum levels of alkaline phosphatase or creatine kinase. Mechanistically, GLU increased intracellular calcium levels and induced calpain-mediated apoptosis. Co-incubation with verapamil-hydrochloride, a surface calcium channel blocker; MDL, a calpain inhibitor; or culturing cells in calcium free media abolished GLU induced increases in intracellular calcium and cytotoxicity. This suggests that calpain and extracellular calcium are functionally important for GLU induced AML cell death. Interrogation of publically available data sets shows that surface calcium channels are significantly (>1.25 fold, $p<0.001$) under-expressed in AML cells compared to normal, suggesting that regulation of calcium through these channels is critical to regulating AML cell viability. In summary, glucopsychosine is a novel therapeutic that selectively induces calpain mediated apoptosis and may be useful in future AML treatments.

ORAL PRESENTATION ABSTRACTS: AFTERNOON SESSION (*Study Leader)

Invited Local Speaker

April Khademi, Department of Biomedical Engineering, School of Engineering, University of Guelph*

Image analysis solutions for the automatic analysis of pathology specimens

Pathology, the analysis of cells and tissues under magnification, is a central discipline in medicine and research, as it provides the definitive diagnosis and helps to understand the etiology of disease. For example, Pathologists use semi-quantitative grading and scoring systems when visually analyzing tissue sections to describe metastatic potential of cancer and the likelihood of response to therapy. Moreover, researchers visually analyze the tissue and cells for the presence of biomarkers, apoptotic cells, necrosis, etc., using both human and animal samples for toxicological studies, pharmaceutical development, biological research, companion diagnostics, and more. Unfortunately, the visual review of tissue slides is a subjective and labourious process, creating variability in the grades or scores, which for large patient cohorts, can take a long time to obtain. With the recent commercialization of digital pathology scanners, it is now possible to digitize tissue specimens and run algorithms on the datasets to automatically

analyze them. Such developments offer objective, quantitative, reliable, and efficient measures that can improve concordance rates among pathologists and researchers, resulting in better patient management and more efficient research studies. The data generated by digital pathology scanners constitutes "bigdata", as the structures that need to be detected are variable and complex and each image can be several gigabytes or terabytes. Specialized algorithms are needed to mine information from these images, that have not been invented before in this new field. This talk explores some of the image analysis research that is being conducted to analyze tissue, and shows how such systems will play a major role in the future of personalized medicine.

Short Talk from Submitted Abstracts

Christian Ternamian, Scott Walsh, Jondavid de Jong, Dorothee Bienzle, Sarah Wootton, Byram Bridle; Department of Pathobiology, University of Guelph*

Oncolytic Rhabdoviruses in Combination with Histone Deacetylase Inhibition Synergistically Kill Murine B Lymphoblastic Leukemia Cells

B-cell acute lymphoblastic leukemia (B-ALL) is a hematological disease characterized by the rapid expansion and metastases of malignant lymphoblasts from the bone marrow. Though conventional treatment can be relatively efficacious in younger patients without central nervous system (CNS) involvement, ALL in adult patients is frequently refractory to current therapies. Furthermore, ALL treatments, including chemotherapy and radiation therapy, significantly increase the risk of developing adverse health effects, including secondary neoplasms, organ toxicities, and reduced cognitive function. Oncolytic virotherapy is an emerging treatment modality that uses oncolytic viruses (OVs) to selectively destroy tumor cells, while sparing healthy cells and generating an anti-tumor immune response. Although a heterogeneous tumor population will often contain cells that retain a quasi-functional antiviral immune response that can impede viral oncolysis, epigenetic-targeted drugs known as histone deacetylase inhibitors (HDIs) can restore sensitivity to OVs in resistant tumor cells. Here, we demonstrate that the newly characterised Maraba MG1 virus and a panel of five HDIs have potent antileukemic effects in vitro. Interestingly, when the HDIs were used in combination with OVs, they synergistically reduced the viability of murine L1210 B-ALL cells. Contrary to the existing paradigm, we hypothesize that OVs may sensitize B-ALL cells to HDI-mediated toxicity. This is supported by data that suggest OVs may be replaced with viral mimics such as imiquimod. Our next objective is to investigate whether combining the Maraba MG1 virus or imiquimod with one of two HDIs (MS-275 or SAHA) can synergistically increase survival in an aggressive murine model of B-ALL.

Short Talk from Submitted Abstracts

J.J.Liu1, S.A.Abdelmagid, L.M.Hillyer, M.Leslie and D.W.L. Ma; Department of Human Health & Nutritional Science, University of Guelph*

Plant- and marine-derived n-3 polyunsaturated fatty acids reduce mammary gland tumor development in MMTV-neu-YD5 mice

Introduction: A healthy diet and lifestyle is critical for the prevention of cancer. Evidence has shown that dietary fatty acids, especially long-chain n-3 polyunsaturated fatty acids (PUFA) play a role in cancer prevention. N-3 PUFA from marine sources, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been shown to exert anti-tumorigenic effects. However, the evidence regarding the role of plant-based n-3 PUFA, or α-linolenic acid (ALA) remains equivocal. Therefore, the present study aims to compare the relative potency of plant versus marine based n-3 PUFA in inhibiting mammary gland tumor development within the MMTV-neu-YD5 mouse model. Experimental Design: Heterozygous MMTV-neu-YD5 males were bred with wild type female FVB mice; the resultant female offspring were maintained on the same parental diet, containing 10% (w/w) fat as either safflower oil alone (control), flaxseed oil alone, or 7 % (w/w) safflower oil plus 3 % (w/w) flaxseed oil or menhaden oil. Mammary glands were palpated for tumors over a 20-week time course, and tumor onset, size and multiplicity were tracked. Tumor tissue and adjacent mammary glands were analyzed for fatty acid composition by gas chromatography. Results: Mice exposed to either 10% flaxseed oil or 3% menhaden oil diets exhibited a reduction in tumor volume as well as an extended tumor latency compared to those fed a 10% safflower oil diet. Data is still being collected for mice fed a 3% flaxseed oil diet. Conclusion: Lifelong exposure to n-3 PUFA, whether from marine or plant sources, can mitigate tumor growth and inhibit mammary carcinogenesis, while the long-chain n-3 PUFA was more potent than plant-derived ALA. Further experiments are warranted to investigate the potential mechanisms of the anti-tumorigenic actions of n-3 PUFA.

Short Talk from Submitted Abstracts

N Weidner[1], A Verbrugghe[1], P Conlon[2], KA Meckling[3], JL Atkinson[4], J Bayle[5], JP Woods[1]; [1]Clinical Studies, [2]Biomedical Sciences, [3]Human Health & Nutritional Sciences, [4]Department of Animal and Poultry Science, University of Guelph; [5]Royal Canin Research Center, Aimargues, France*

Dietary vitamin D intake and vitamin D status in canine cancer patients

Despite high cancer rates, there is little literature about nutritional risk factors and management of cancer in dogs. Low vitamin D intake and low vitamin D status have been linked to increased risk and decreased survival in many human cancers. This study examined the dietary vitamin D intake and vitamin D status of dogs with cancer in comparison to healthy dogs. Client owned dogs presenting to the Animal Cancer Centre at the Ontario Veterinary College with osteosarcoma (n=15), lymphoma (n=29) and mast cell tumors (n=25) were enrolled. Owners provided dietary information and a sample of the dog's food for calculation of each dog's vitamin D intake. Blood samples were collected and analyzed for 25-hydroxyvitamin D (25OHD), ionized calcium, PTH and PTHrP. This was repeated for a group of 18 healthy, control dogs. Statistical analysis was done with SAS 9.3 using an ANCOVA. Median plasma 25OHD levels were significantly higher in healthy dogs (135.67 nmol/L) than in those with osteosarcoma (92.55 nmol/L, p = 0.0012), lymphoma (100.0 nmol/L, p =0.0027) and mast cell tumors (107.87 nmol/L, p=0.0256). This relationship existed regardless of each dog's vitamin D intake. PTH, iCa and PTHrP did not differ significantly between healthy dogs and those with

cancer. The observed difference in 25OHD between healthy dogs and those with mast cell tumors is consistent with a previous report. This is the first study to examine 25OHD concentrations in dogs with osteosarcoma and lymphoma. The significantly lower vitamin D status in dogs with cancer compared to healthy dogs warrants further investigation. A prospective cohort study would help determine whether decreased plasma 25OHD concentrations are associated with increased cancer risk, or whether cancer development is associated with alterations in vitamin D metabolism. Dogs with cancer in this study are being followed to determine effects of treatment on 25OHD levels, as well as associations between 25OHD and survival.

Invited Local Speaker

Dr. Valerie Poirier, OVC HSC Animal Cancer Centre, University of Guelph*
Radiation, new with old

Radiation therapy has been used to treat cancer patients for the last 120 years. In that time, huge leaps in technology have been achieved. At the Animal Cancer Centre, we are now privileged to have the most advanced technology (Clinac iX) to treat our patients and a huge body of research will be coming our way in the future. After highlighting the capability of our new machine, I will present the results of multiple studies that I have been involved with in the last 5 years both here and at the Brisbane Veterinary Specialist Centre in Australia. The first two studies were looking at new radiation protocols to treat canine soft tissue sarcomas and feline oral squamous cell carcinomas. The following study was funded by Pet trust and evaluated different bolus material to use for electron beam therapy on the canine limb. The final study was the use of radiation to deplete stem cells in Brahman bull testes prior to injection of Angus Bull stem cells.

LUNCH & POSTER SESSION 12:00-1:30

OVC LLC Room 1707 B & C

Posters will be displayed all day; authors please attend your poster 12:30-1:30

1) Inhibition of HSP70 for the Treatment of Canine Osteosarcoma
Jonathan Asling, Brooke Fraser, Anthony J. Mutsaers; Department of Biomedical Science, University of Guelph*

2) Investigating the effectiveness of Akt1 inhibitor on non-small cell lung tumour supression in-vitro
Ritesh Briah, Roger Moorehead, S. Elizabeth Franks; Department of Biomedical Science, University of Guelph*

3) Changes In matrix metalloproteinase expression in SN-38-resistant colorectal cancer cells
Spencer I.T. Berg, Murray J. Cutler, Jonathan Blay; Department of Pharmacy, University of Waterloo*

4) Evaluating drug resistance in colorectal cancer stem cells
Stacey J. Butler, Nathan Farias, Brenda L. Coomber; Department of Biomedical Sciences, University of Guelph*

5) ICCI Clinical Research: Companion Animal Tissue Sample Bank and Clinical Trials in the Mona Campbell Centre for Animal Cancer
Astrid Cuncins-Hearn, Brenda Coomber, Paul Woods*; Institute for Comparative Cancer Investigation, University of Guelph*

6) The Influence of Isoform Type and Oxygen Levels in the Angiogenic Response of Endothelial Cells to TGFβ
Meghan Doerr, Alicia Viloria-Petit; Department of Biomedical Sciences, University of Guelph*

7) The Effects of Chemotherapy Scheduling on Ovarian Function and Female Fertility
Jacqueline Dynes, Jim Petrik; Department of Biomedical Sciences, University of Guelph*

8) The response of canine osteosarcoma cells to Doxorubicin chemotherapy after siRNA-mediated silencing of Prkar1a expression
Gallienne J[1], Petrik N, Liu J[1], Wood G[1]; [1]Department of Pathobiology, [2]Department of Biomedical Sciences, University of Guelph

9) Effects of 3-bromopyruvate on human colorectal cancer metabolism
Nelson Ho, Brenda L. Coomber; Department of Biomedical Sciences, University of Guelph*

10) Inhibition of glucose uptake through sodium-glucose transporters (SGLT) demonstrates metabolic effects on epithelial ovarian cancer (EOC) cells, independent of GLUT-mediated glucose uptake
Lisa Kellenberger, Jim Petrik; Department of Biomedical Sciences, University of Guelph*

11) Investigating Nkd1's activation and role in Wnt signaling
Roman Kondra, Terry Van Raay; Department of Molecular & Cellular Biology, University of Guelph*

12) AVO: a novel inhibitor of fatty acid oxidation that induces selective leukemia cell death
Lee EA[1], Angka L[1], Rota SG[1], Hurren R[2], Wang XM[2], Gronda M[2], Minden M[2], Mitchell A[3], Datti A[4,5], Wrana, J[4], Joseph JW[1], Quadrilatero J[3], Schimmer AD[2], Spagnuolo PA[1]; [1]School of Pharmacy, [3]Department of Kinesiology, University of Waterloo, [2]Princess Margaret Cancer Center, OCI, Toronto; [4]SMART laboratory for High-Throughput Screening Programs, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto; [5]Department of Agricultural, Food and Environmental Sciences, University of Perugia, 06100, Perugia Italy

13) Canine mast cell tumour (MCT) cells treated with itraconazole and toceranib exhibit decreased proliferation rates and altered posttranslational modifications of KIT and VEGFR2.
Sean Masson, Jennifer Thompson, Brenda L. Coomber; Department of Biomedical Sciences, University of Guelph*

14) Perioperative Management and Outcome of Bilateral Adrenalectomy in 9 dogs
Michelle Oblak[1], Nicholas Bacon[2], Jen Covey[3]; [1]Ontario Veterinary College, University of Guelph; [2]College of Veterinary Medicine, University of Florida, Gainesville, FL, USA; [3]Pittsburgh Veterinary Specialty and Emergency Center, Pittsburgh, PA, USA*

15) In Vitro Effects of Wee1 Kinase Inhibition on Chemotherapy and Radiation Response in Osteosarcoma Cell Lines
Steven G. Patten, Sarah Darakhshan, Devan E. Thompson, Kaela Shaw, Carl R. Walkley, Anthony J. Mutsaers; Department of Biomedical Sciences, University of Guelph*

16) Regulation of Noxa expression by miR-23a in heat-stressed cells
Rabih Roufayel, Donald Johnston, Dick D. Mosser; Department of Molecular & Cellular Biology, University of Guelph*

17) miRNA regulation of Puma translation in heat-shocked cells
Rebecca Rumney, Richard D. Mosser; Department of Molecular & Cellular Biology, University of Guelph*

18) The Effects of 3TSR and Combinational Metronomic Chemotherapy on Epithelial Ovarian Cancer
Samantha Russell[1], Jack Lawler[2], Jim Petrik[1]; [1]Department of Biomedical Sciences, University of Guelph, [2]Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts, USA*

19) Treating high-grade glioma with oncolytic virotherapy and histone deacetylase inhibitors
Zafir Syed, Scott Walsh, Byram W. Bridle; Department of Pathobiology, University of Guelph*

20) The Effects of 3TSR Fusion Proteins on Ovarian Cancer

Simone ten Kortenaar, Jack Lawler[2], Jim Petrik[1]*; [1]Biomedical Sciences, Ontario Veterinary College, University of Guelph; [2]Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts, USA*

21) The development of recombinant *Parapoxvirus ovis* (ORFV) for use in oncolytic virotherapy.

Jacob P. van Vloten, Jondavid de Jong and Sarah K. Wootton; Department of Pathobiology, University of Guelph*

22) Delivery of toxic topoisomerase mutant alleles by DNA ministrings for ovarian cancer gene therapy

Shirley Wong, Nafiseh Nafissi, Chi Hong Sum, Roderick Slavcev; School of Pharmacy, University of Waterloo*

POSTER ABSTRACTS (*study leader)

1) Inhibition of HSP70 for the Treatment of Canine Osteosarcoma

Jonathan Asling, Brooke Fraser, Anthony J. Mutsaers; Department of Biomedical Science, University of Guelph*

Osteosarcoma (OSA) is the most common primary bone cancer in humans and canines. Despite the ability of chemotherapy treatment to prolong survival, canine patients continue to have a poor long-term prognosis. Heat shock proteins (HSPs) typically function as molecular chaperones. However, HSP production is rapidly induced following various cellular stresses including anticancer treatment. Under these stressful conditions, HSPs can prevent apoptosis. One HSP, HSP70, has been identified as a potential marker for high-grade canine OSA. The presence of HSP70 is related to the development of grade III lesions and conversely the absence of HSP70 has been associated with longer survival times. Treatment of canine OSA cell lines with VER155008, an n-terminal ATPase inhibitor of HSP70, reduced cell viability. Furthermore, this small molecule inhibitor increased apoptosis and decreased the number of colonies formed in clonogenic survival assays. When combined with the chemotherapeutic agent doxorubicin, HSP70 inhibition increased caspase-3 cleavage and cellular death to levels in excess of that achieved by doxorubicin alone. However, in response to HSP70 inhibition, canine OSA cells up-regulate HSP70 and its protein family member GRP78, which becomes localized throughout the cytoplasm and nucleus. Up-regulation of HSP70 was not reliant upon AKT activity and, unlike GRP78, was not observed when murine transgenic OSA cell lines were treated with VER155008. Mechanisms responsible for increased GRP78 production have yet to be elucidated. Given the role of HSP70 and GRP78 in the prevention of apoptosis, further studies are required to determine if their expression patterns are detrimental to doxorubicin treatment.

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2) Investigating the effectiveness of Akt1 inhibitor on non-small cell lung tumour supression in-vitro

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Akt signaling molecules are part of the PI3K pathway and are involved with cell growth, proliferation, metabolism, survival and migration. Increased Akt activation has been shown in lung cancer cells and is associated with poor prognosis. While it was previously believed that all 3 isomers of Akt have similar roles in tumour progression, emerging research has shown divergent roles. Our lab was previously able to demonstrate that Akt 1 knockout mice formed fewer tumours than Akt2 knockout mice and appeared to be less invasive as well. These results indicate that Akt1 targeted therapy may be more effective in lung tumour suppression than the pan-Akt inhibitors currently undergoing clinical trials. To investigate these findings further, we decided to gather preliminary data through an in-vitro model. Four different cell lines were used to generate cell survival curves to determine EC50 dosages of Akt1, Akt2, and pan-Akt inhibitors. The data gathered showed that the Akt1 inhibitor was more potent in human cancer cells than the other two inhibitors. Currently, proliferation and apoptosis rates are being measured by immunofluorescence with Phospho-Histone H3 and Cleaved Caspase-3 antibodies respectively. The initial data shows a trend of increased proliferation at 24 hours with Akt1 inhibition in a dose dependent manner, which may indicate cellular compensation in a last attempt to survive, however data must be gathered at an earlier time point to validate these claims. The next steps include isomer specific protein analysis through western-blotting coupled with densitometry. If significant differences are found between treatments then chemotherapy coupled treatment with carboplatin and cisplatin will be investigated.

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3) Changes In matrix metalloproteinase expression in SN-38-resistant colorectal cancer cells

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The matrix metalloproteinases (MMPs) are a complex family of zinc-dependent proteolytic enzymes, which collectively are capable of degrading all components of the extracellular matrix (ECM). The ECM provides structure and support for normal tissues, and acts both as a barrier to and support for cancer cell invasion and metastasis. MMPs are therefore believed to play an important role in regulating the rate of cancer progression. However, the repeated failure of MMP inhibitors in clinical trials, as well as recent discoveries of novel MMP activities and localizations, has led to a re-evaluation of the roles of MMPs to the cancer process. In colorectal cancer (CRC) the outlook following disease relapse after surgery and initial chemotherapy is poor, due both to drug resistance and further dissemination of disease. We investigated the involvement of MMPs and related molecules in this context. We have generated a series of derivatives of the human CRC cell line HT29 that are resistant to SN-38, the active metabolite of the chemotherapeutic agent irinotecan. The principal representative cell line, HT29-S, has a slower proliferation rate than parental HT29 cells, and yet forms denser outgrowths in monolayer culture. The abundance and localization of several MMPs and tissue inhibitors of metalloproteinases (TIMPs) have been assessed in both HT29 and HT29-S cells by western blotting of cytosolic and nuclear protein fractions, and by immunofluorescence. HT29-S cells

showed a lower expression of MMP1, MMP7, and MMP9, but also TIMP1 and TIMP2, compared to their parental counterparts, with some differences in cellular distribution. In particular, MMP1 gave a strong nuclear signal by both methods in HT29 cells, which was strongly reduced in HT29-S. A reduction in MMP expression will allow for an increased accumulation of ECM components. This may act through integrin receptors to activate survival pathways and contribute to the chemoresistance observed in HT29-S cells, and may also facilitate the spread of cancer cells by refining the ECM framework in advanced CRC.

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4) Evaluating drug resistance in colorectal cancer stem cells

Stacey J. Butler, Nathan Farias, Brenda L. Coomber; Department of Biomedical Sciences, University of Guelph*

The effectiveness of chemotherapy for advanced colorectal cancer is impacted by a lack of selectivity, the development of drug resistance, and the overall heterogeneity existing within a tumour. Cancer stem cells (CSCs) are a subpopulation of tumour cells that exhibit stem-like characteristics, including the ability to self-renew and differentiate, and are thought to be responsible for tumour recurrence due to their ability to evade current treatments. This project explores the responses of CSCs to chemotherapy and identifies therapeutic targets to eradicate the CSC population. We used serial culture to isolate stem-like cells from two human colorectal cancer cell lines, HCT116 and SW480, based on their ability to form colonospheres in serum-free 3D culture. A limiting dilution analysis was performed to compare the colonosphere forming potential of the stem-like and respective parental cells. Stem-like cells showed a significant increase in the ability to form colonospheres at low densities compared to parental cells. Crystal violet assays for cell number were used to generate dose-response curves from the parental cells in monolayer culture, against three commonly employed chemotherapeutic drugs; 5-fluorouracil, cisplatin and epirubicin. 3D treatments were then performed and cell viability was analyzed via PrestoBlue, on the stem-like and parental colonospheres using the IC_{10}, IC_{50} and IC_{90} doses derived from monolayer treatments. The stem-like cells exhibited significant resistance to all chemotherapeutics, with the exception of HCT116 stem-like cells demonstrating sensitivity to 5-fluorouracil in a dose-dependent manner. Chemotherapy treatment affected the cellular adhesion of the colonospheres resulting in significant dissociation at high doses. The degree of dissociation was greater for the parental cell colonospheres than the stem-like cell colonospheres in the majority of treatments. Future experiments will further characterize these stem-like cells based on known colorectal CSC markers, as well as explore the observed changes in adhesion as a response to chemotherapy.

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5) ICCI Clinical Research: Companion Animal Tissue Sample Bank and Clinical Trials in the Mona Campbell Centre for Animal Cancer

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Pets, primarily dogs and cats, are presented to the Mona Campbell Centre for Animal Cancer at OVC for diagnosis and treatment of naturally occurring spontaneous cancers. To give these pets

the benefit of cutting-edge research and novel treatments (which may translate to human cancer care) and to help cancer researchers better understand the biology of cancer, the Institute for Comparative Cancer Investigation (ICCI) engages in clinical research including the Companion Animal Tissue Sample Bank (CATSB) and clinical oncology trials. The CATSB is a unique biospecimen resource, as the only veterinary tumour bank in Canada, collecting cancer and noncancerous tissue, serum, plasma, and urine samples from pets having surgery to remove their tumours at the OVC Health Sciences Centre (OVC HSC). With owner consent, samples are collected within one hour of resection and preserved as fresh frozen samples or in stabilizing reagents (RNA Later ®, OCT compound (Tissue Tek®) and stored in a -80 C freezer. Clinical information including histopathology results and treatment details are linked to each sample. Presently, there are 2,398 samples collected from 271 cases representing both common and rare tumour types. Current ICCI Clinical Trials include investigating a new biomarker assay as a predictor of chemotherapy efficacy (*Using ribosomal RNA disruption (RNA Disruption Assay (RDA)) as a predictor of early relapse in canine lymphoma*). A trial is investigating the relationship between vitamin D status and dietary intake in cancer-bearing dogs versus healthy dogs (*Vitamin D status in canine cancer patients and the relationship with dietary vitamin D intake*). A microRNA trial is correlating expression of microRNA to outcome (*Diagnostic and prognostic utility of microRNA in blood and tissues of dogs with multicentric lymphoma*). Another trial is investigating the effect of tyrosine kinase inhibitor (TKI) toceranib (Palladia®) therapy on cytokines. http://ovc.uoguelph.ca/icci

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6) The Influence of Isoform Type and Oxygen Levels in the Angiogenic Response of Endothelial Cells to TGFβ

Meghan Doerr, Alicia Viloria-Petit; Department of Biomedical Sciences, University of Guelph*

Transforming growth factor-beta (TGFβ) is often overexpressed in solid tumors, where it potentially plays a role in tumor angiogenesis, but this process is not fully understood. Previous studies demonstrated a cross-talk between TGFβ and vascular endothelial growth factor (VEGF), whereby low concentrations of TGFβ1 (up to 0.5 ng/mL) cooperate with VEGF in stimulating an angiogenic response in cultured endothelial cells (ECs). However, it is still unclear whether this holds true for other TGFβ isoforms and whether the tumor's oxygen level can further influence this response. TGFβ isoforms 1 and 2 play non-over lapping roles in vascular development and cancer. Variations in oxygen levels are common in tumors, and low oxygen/hypoxia is a potent inducer of angiogenesis. Thus, we hypothesize TGFβ's capacity to induce an angiogenic response depends on the type of TGFβ isoform and oxygen level of the surrounding microenvironment. To begin testing this hypothesis we characterized TGFβ response in two EC lines: bovine aortic endothelial cells (BAEC) and human pulmonary endothelial cells (HPMEC). Using immunoblotting/immunofluorescence we demonstrate expression of VEGF and TGFβ receptors and activation of canonical TGFβ signaling (mediated by Smad2/3) in response to TGFβ1/2 in these cell lines. We also show that both cell lines form tube-like structures in response to TGFβ1 and VEGF when grown on Matrigel™. This demonstrates the appropriateness of our cell lines for investigating the role of oxygen levels in modulating TGFb's response in ECs, which is necessary to improve our understanding of TGFβ's role in tumor angiogenesis.

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7) The Effects of Chemotherapy Scheduling on Ovarian Function and Female Fertility
Jacqueline Dynes, Jim Petrik; Department of Biomedical Sciences, University of Guelph*

Chemotherapy can cause early menopause or infertility in young women and have a profound negative impact on the quality of life of young female cancer survivors. The irreversible long-term ovarian damage caused by cytotoxic drugs is due to depletion of the limited primordial follicle (PMF) population. One potential mechanism of chemotherapy-induced loss of this resting PMF pool is by their increased activation in response to damage to the highly proliferative growing follicle population. Conventional Maximum-Tolerated Dose (MTD) chemotherapy is toxic to normally proliferative healthy cells in addition to cancer cells, causing serious adverse effects and the need for a recovery period between dosing intervals. The alternative Low-Dose Metronomic (LDM) scheduling approach that involves frequent low-dose chemotherapy administration has been associated with reduced short-term side effects. We believe that LDM scheduling will also have reduced detrimental effects on ovarian function and female fertility. We treated mice with equivalent cumulative doses of cyclophosphamide by either LDM or MTD scheduling, their ovarian cycles were synchronized, and ovaries were collected at various stages of follicle development. Ovaries were assessed for morphometric changes and markers of cell proliferation and apoptosis. While the ovarian follicle dynamics reflected that of normal controls following LDM treatment, increased apoptosis was detected in growing ovarian follicles after MTD treatment and this was shortly followed by elevated numbers of early growing follicles. The proportion of healthy growing to early atretic follicles was also only significantly reduced following MTD administration. These data suggest that LDM scheduling is associated with reduced PMF wasting as a result of decreased toxicity to growing follicles and may reveal a novel benefit of LDM chemotherapy scheduling that could be implicated in fertility preservation for young females undergoing chemotherapy.

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8) The response of canine osteosarcoma cells to Doxorubicin chemotherapy after siRNA-mediated silencing of Prkar1a expression
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Companion animals, such as the dog, can spontaneously develop cancers just like humans. It is anticipated that cancer research using both species may provide a better understanding of tumorigenesis, and reveal novel therapeutic targets and prognostic markers for the disease. Canine osteosarcoma (OSA) is an aggressive neoplasm composed of malignant osteoblasts and this type of cancer shows remarkable similarities to human OSA. In addition to similar clinical profiles, both species have primary tumours displaying many of the same DNA amplifications and deletions, and gross chromosomal abnormalities. Prkar1a, known as the R1 alpha regulatory subunit of cyclic AMP-dependent protein kinase A, is deleted in a subset of mouse bone cancer and is under expressed at the RNA level in human bone cancers that have the best post-chemotherapy response. We assessed Prkar1a expression in canine bone cancer at the protein level and found that low expression correlated with longer post-chemotherapy survival. Based on this finding, we hypothesized that inhibiting the expression of Prkar1a in canine bone cancer cells would increase their sensitivity to chemotherapeutic drugs. In this study, we measured the

chemotherapeutic response of four canine bone cancer cell lines with naturally low, naturally high, or experimentally reduced Prkar1a expression after exposure to Doxorubicin. Transient experimental inhibition of Prkar1a was induced by Lipofectamine 3000-mediated transfection of small interfering RNA (siRNA). Overall, Prkar1a may provide a potential therapeutic target to sensitize bone cancer cells to chemotherapy, leading to enhanced treatment efficacy and improved patient survival for both humans and animals.

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9) Effects of 3-bromopyruvate on human colorectal cancer metabolism

Nelson Ho, Brenda L. Coomber; Department of Biomedical Sciences, University of Guelph*

The Warburg Effect describes the widely observed metabolic phenotype of cancer cells and their heavy reliance on the glycolytic pathway for ATP production regardless of oxygen tension. 3-bromopyruvate (3BP) is a promising anti-cancer compound capable of targeting critical energy pathways in cancer cells. Its primary target, hexokinase II (HKII), is a glycolytic enzyme often overexpressed in tumors as a result of their unique metabolic signature. Although 3BP has been shown to effectively kill cancer cells, the underlying mechanisms have not been thoroughly investigated. This project aims to explore the processes by which 3BP exhibits its action on human colorectal (CRC) cells. A panel of CRC cell lines was initially screened for the expression of different HK isoforms. A subset of these cell lines were used to further investigate the impact of 3BP on survival pathways and metabolic output. HCT116 and CaCo2 cells expressed low levels of HKII while SW480 and DLD-1 cells highly expressed HKII. Cells were treated with 0 – 50 μM 3BP for various time points for subsequent analysis via western blot. Changes in survival signaling pathways following 3BP treatment occurred within 15 minutes of treatment. AKT phosphorylation increased in a dose-dependent manner and was residue-specific. Through real-time measurement of cellular bioenergetics, we examined the respiration rates of HCT116 cells following 24 hr 3BP treatment. Upon examination of intermediary metabolism, we observed significant changes in glucose consumption and lactate production only in the cells treated with 50 μM 3BP. Future experiments will further elucidate the mechanisms by which 3BP is able to induce cancer cell death. This study will provide additional support for the potential use of 3BP as an anti-cancer agent against human colorectal cancer.

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10) Inhibition of glucose uptake through sodium-glucose transporters (SGLT) demonstrates metabolic effects on epithelial ovarian cancer (EOC) cells, independent of GLUT-mediated glucose uptake

Lisa Kellenberger, Jim Petrik; Department of Biomedical Sciences, University of Guelph*

Tumor cells have an abnormally high glycolytic rate that requires a high volume of glucose, and thus an enhanced ability to take up glucose from the environment. Preliminary *in vitro* work in our lab has shown that mouse epithelial ovarian cancer (EOC) cells exposed to chronically high glucose become more adept at metabolizing available glucose and more aggressive than cells in normal glucose. *In vivo*, we have shown that elevated glucose alone is sufficient to increase tumor growth and decrease overall survival. We have demonstrated the presence of both active (SGLT) and passive (GLUT) glucose uptake transporters in mouse and human EOC cells. We hypothesize that because of their ability to pump against a concentration gradient, SGLTs will be

particularly important to EOC cell metabolism when the capacity for passive transport is exhausted. EOC cells were treated with the pharmacological inhibitors phlorizin (PZ) or Cytochalasin B (CB) to inhibit function of the SGLTs or GLUTs respectively, and viability and functional assays were performed. We found that PZ was more effective than CB at reducing glucose uptake in hyperglycemic environments. Cells grown in chronically high glucose were also more sensitive to the effects of PZ, which led to a dose-dependent increase in viability. Treatment of mouse EOC cells with PZ inhibited wound closing in a scratch assay, although interestingly, PZ promoted cell migration in a Boyden chamber. PZ did not affect cell number, suggesting that these results are independent of a change in the balance of proliferation/apoptosis. SGLTs appear to partially mediate EOC cell metabolism and motility in the presence of high levels of glucose. By targeting these transporters, we may be able to decrease the aggressiveness of EOC cells *in vitro* and *in vivo*.

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11) Investigating Nkd1's activation and role in Wnt signaling

Roman Kondra, Terry Van Raay; Department of Molecular & Cellular Biology, University of Guelph*

The Wnt signaling pathway is a highly conserved pathway critical for development and homeostasis of stem cells. Aberrant Wnt signaling causes developmental defects and disease, most notably cancer, as over 90% of colorectal cancers (CRC) contain mutations in this signaling pathway. β-catenin is a central molecule of Wnt signaling, translocating into the nucleus to activate Wnt target genes. One of these transcriptional targets, nkd1, is involved in a negative feedback regulatory loop yet little is known about its activation or mode of action. Studies involving zebrafish, drosophila and in vitro assays have shown Nkd1 expression and function to be dependent on Wnt activity and that NKD1 interacts with several proteins yet we don't know how these interactions affect NKD1 or Wnt signaling. In zebrafish, NKD1 inhibits Wnt signaling by binding and preventing nuclear accumulation of β-catenin yet NKD1 function is dependent on membrane localization. I hypothesize that NKD1 becomes activated at the membrane, and this activation is dependent on the binding of a Wnt ligand to its receptors. Nkd1 is upregulated in a large number of CRC's yet is insufficient to antagonize Wnt signaling. CRC's have mutations constitutively activating the Wnt pathway independent of a Wnt ligand. It is possible that without a Wnt stimulus, Nkd1 may be unable to abrogate Wnt signaling. To test this hypothesis, I used cancerous and normal mammalian cell lines to look at the distribution of NKD1 and determine if localization is dependent on Wnt signaling. My results have shown that under Wnt stimulus the distribution of Nkd1 changes and interaction between NKD1 and B-catenin increases. In addition the Wnt ligand seems to be hyper-activating the Wnt signaling pathway in constitutively active cancer cells. Understanding Nkd1's function in the Wnt pathway, especially in diseases such as colorectal cancers, will ultimately help us develop better therapies.

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12) AVO: a novel inhibitor of fatty acid oxidation that induces selective leukemia cell death

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Acute myeloid leukemia (AML) is a hematological malignancy with devastating patient outcomes. In adults (>60), 2-year survival rates are less than 10%. To identify potential novel AML therapeutics, we performed a high-throughput chemical screen where avocado lipid A (AVO), an avocado derived compound, was identified to have anti-leukemia activity. AVO reduced the viability of primary AML patient samples at 1.5-5μM but had no effect on normal peripheral blood stem cell viability at 20μM. Furthermore, AVO selectively reduced the clonogenic growth of AML progenitor and stem cells with no effect on normal hematopoietic stem cells. Mechanistically, AVO targets the mitochondria and inhibits fatty acid oxidation. This results in decreased NADPH and ROS-mediated leukemia cell death through the release of pro-apoptotic mitochondrial proteins, AIF and cytochrome c. In summary, AVO is a novel therapeutic that selectively induces leukemia cell death through the inhibition of fatty acid oxidation and may be useful for the future treatment of AML.

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13) Canine mast cell tumour (MCT) cells treated with itraconazole and toceranib exhibit decreased proliferation rates and altered posttranslational modifications of KIT and VEGFR2.

Sean Masson, Jennifer Thompson, Brenda L. Coomber; Department of Biomedical Sciences, University of Guelph*

Canine mast cell tumours (MCTs) are a common skin malignancy in dogs, largely driven by mutations in the c-kit gene. Tumours show KIT dysregulation, and the cancer cells signal through PDGFRs and VEGFRs. High grade/metastatic MCTs are resistant to conventional chemotherapeutics, thus canine MCT cells represent a valuable model for KIT dysregulation in human cancers. Better understanding of aberrant KIT (and other RTK) signalling in these cells could therefore improve design and use of targeted therapies for human cancers driven by c-kit and other mutations. Canine MCTs are currently treated with the TKIs toceranib (Palladia) or masitinib (Masivet), with mixed results. Itraconazole, an anti-fungal agent shown to interfere with VEGFR2 glycosylation and hence signalling, may also affect KIT in these cells. The effect of itraconazole on two MCT cell lines (MCT1 and MCT2) was evaluated individually and in combination with RTK inhibitors. Cell proliferation was quantified in response to itraconazole (0, 100, 200, 400, 800 nM), masitinib (0, 50, 100, 200, 400 nM) and toceranib (1.0 μM) treatment. Masitinib had no inhibitory effect on proliferation in either cell lines. However, itraconazole inhibited cell proliferation of both cell lines, leading to a static cell population compared to control. Combination treatment with toceranib, and to a lesser extent masitinib, significantly lowered MCT-2 cell numbers after 48 hours of treatment. Western blots for both cell lines showed reduced levels of phosphorylated KIT and VEGFR2 in response to itraconazole treatment. More importantly, a shift towards lower molecular weight native proteins was also seen in MCT-2 cells, indicating itraconazole may interfere with receptor glycosylation in these cells. Additionally, immunoprecipitation experiments show altered KIT glycosylation after itraconazole treatment. Itraconazole should therefore be considered a prospective novel therapeutic, warranting further investigation as a treatment for cancers driven by KIT and VEGFR2 dysregulation.

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14) Perioperative Management and Outcome of Bilateral Adrenalectomy in 9 dogs

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Bilateral adrenalectomy in dogs has been infrequently described in the literature with a 21-29% perioperative mortality reported. The purpose of this study was to report cases of bilateral adrenalectomy and describe the perioperative care, postoperative management, and long-term outcome of these patients. Medical records from the University of Florida College of Veterinary Medicine Small Animal Hospital were reviewed. Nine dogs were identified that had undergone bilateral adrenalectomy. Preoperative evaluation revealed suspected HAC in 6 cases. In 2 dogs the LDDS was consistent with adrenal dependent HAC. In 3 dogs, no clinicopathologic changes could be attributed to the adrenal disease but in 2 of these dogs a pheochromocytoma was suspected. Surgery was uncomplicated in most cases, with minimal perioperative morbidity in eight cases. All dogs received IV dexamethasone SP (median 0.20mg/kg, range 0.02-0.40mg/kg) intraoperatively. Eight dogs received IM DOCP (median 2.15, range 1.9-2.4mg/kg) intraoperatively. Histopathologic diagnoses included adrenocortical tumor (11), pheochromocytoma (6), and adrenocortical atrophy (1). One dog died perioperatively and the remainder died due to causes unrelated to their original tumor. Postoperative management of hypoadrenocorticism included oral prednisone + IM DOCP (6), oral prednisone + fludrocortisone (1) and oral fludrocortisone alone (1). The median survival time of all dogs was 525 days. No dogs had evidence of metastatic disease at the time of death and no dog died as a result of complications associated with adrenocortical insufficiency. Based on these findings, bilateral adrenalectomy should be considered in dogs with bilateral adrenal disease regardless of the suspected underlying adrenal pathology. The perioperative mortality may be lower than previously reported, and management of postoperative hypoadrenocorticism is both achievable and straightforward.

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15) In Vitro Effects of Wee1 Kinase Inhibition on Chemotherapy and Radiation Response in Osteosarcoma Cell Lines

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Cell cycle checkpoint responses may reduce efficacy of DNA damaging chemotherapy and radiation treatments by halting cell cycle progression. The p53 response during the G1/S phase is an important example. Dysfunction of the p53 pathway is common in many cancers and may be fundamental to osteosarcoma pathogenesis. Cells deficient in p53 function rely on alternate checkpoint kinases, such as wee1 to regulate cell cycle progression and repair DNA damage to avoid mitotic catastrophe-induced cell death. The purpose of this study was to investigate whether inhibition of wee1 would sensitize osteosarcoma cells to chemotherapy and radiation treatment. Canine and mouse osteosarcoma cell lines were utilized. The mouse cell lines were isolated from tumors of transgenic mice with targeted p53 knockout in the pre-osteoblast lineage. Drug choices included doxorubicin and carboplatin chemotherapy and the small molecule inhibitor of wee1 kinase MK-1775. Radiation was delivered by a Co-60 source or linear accelerator. Cell viability, colony formation and phospho-cdc2 assays were conducted to assess single agent and combination drug treatment efficacy. MK-1775 caused a dose-dependent

inhibition of phospho-cdc2 in transgenic mouse osteosarcoma cells deficient for p53. Use of MK-1775 in nanomolar concentrations as a single agent and in combination with doxorubicin, carboplatin, or 2-8Gy of radiation significantly reduced viability and colony formation in some, but not all cell lines tested. Preliminary results suggest there may be a role for wee1 inhibition to improve osteosarcoma treatment responses to chemotherapy and/or radiation.

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16) Regulation of Noxa expression by miR-23a in heat-stressed cells

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Protein damaging stress, such as exposure to elevated temperature, can activate a process of cellular destruction known as apoptosis but also induces the synthesis of heat shock proteins including HSP70, which can protect cells from stress-induced apoptosis. Apoptosis is regulated by pro-apoptotic members of the Bcl-2 family (Bax and Bak) that oligomerize to form channels in the mitochondrial outer membrane permitting the release of pro-apoptotic caspase-activating factors which ultimately cause the proteolytic dismantling of the dying cell. Anti-apoptotic members of the Bcl-2 family (Bcl-2, Mcl-1) prevent apoptosis by antagonizing Bax/Bak oligomerization. Pro-apoptotic BH3-only proteins act upstream of the anti-apoptotic members to inhibit their ability to block Bax and Bak activation. In this study we sought to determine the mechanism responsible for the heat-induced accumulation of the BH3-only protein Noxa (PMAIP1). Analysis of transcript levels by qRT-PCR in cells exposed to hyperthermia revealed that increased Noxa mRNA levels were correlated with a corresponding decrease in miR-23a levels. Interestingly, cells overexpressing HSP70 maintained higher levels of miR-23a after heat shock and accumulated lower levels of Noxa mRNA and protein. A direct role for miR-23a in regulating Noxa expression was demonstrated by miR-23a overexpression and shRNA mediated-knockdown as well as luciferase reporter assays in transiently transfected HeLa cells. Stable overexpression of miR-23a in the acute lymphoblastic T cell line PEER resulted in reduced basal and heat-induced levels of Noxa mRNA and protein and significantly inhibited heat-induced apoptosis. Additionally, stable overexpression of an shRNA targeting miR-23a in the lymphoma cell line U937 produced stable knockdown of miR-23a and resulted in increased Noxa mRNA and protein and an increased sensitivity to heat-induced apoptosis. These results demonstrate the novel finding that hyperthermia affects the abundance of a microRNA that targets the expression of a pro-apoptotic protein and that HSP70 protects cells from heat-induced apoptosis by preventing the loss of this microRNA.

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17) miRNA regulation of Puma translation in heat-shocked cells

Rebecca Rumney, Richard D. Mosser; Department of Molecular & Cellular Biology, University of Guelph*

This thesis is an investigation of heat-induced apoptotic cell death. Apoptosis is a highly regulated means of eliminating damaged cells. Deregulated apoptosis is responsible for a large number of human diseases including neurological disorders, stroke, as well as autoimmune disorders and cancer where apoptosis fails to occur. Stress-induced apoptosis can be caused by various conditions including elevated temperature, a model system for studying the

consequences of protein misfolding. Hyperthermia induced apoptosis is mediated by the Bcl-2 family of proteins. This family consists of both pro-apoptotic and anti-apoptotic members that regulate mitochondrial integrity. Apoptosis is controlled by the BH3-only pro-apoptotic members, which cause the oligomerization of Bak/Bax in the outer mitochondrial membrane, resulting in the release of cytochrome c from the mitochondrial inter-membrane space to the cytosol and subsequent activation of caspase 9 and 3 leading to cell destruction. Our examination of the effect of hyperthermia on Bcl-2 family protein expression found that the BH3-only protein Puma is rapidly depleted following exposure to hyperthermia while at the same time levels of Puma mRNA increase. This suggests that post-transcriptional mechanisms regulate the translation of Puma mRNA in heat-shocked cells. The goal of this project is to examine the mechanism controlling Puma expression following heat shock, specifically to assess the role of miRNA-mediated inhibition of Puma translation. Currenty, miRNAs have been shown to target many apoptotic regulators, including Puma. By targeting and binding Puma mRNA, selected miRNAs may cause translational suppression. Our study explores three miRNAs that are predicted to target the Puma 3' untranslated region and show increased expression after hyperthermia. This elevated expression of miRNAs coincides with the decreased expression of Puma protein after heat-shock. Aberrant expression of Bcl-2 family proteins, including Puma, contributes to carcinogenesis and alters sensitivity to chemotherapeutic agents. Understanding the mechanisms regulating Puma expression may provide a novel approach for altering stress resistance in cancer cells through manipulation of miRNA expression.

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18) The Effects of 3TSR and Combinational Metronomic Chemotherapy on Epithelial Ovarian Cancer

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Epithelial ovarian cancer (EOC) is the most lethal gynecological cancer affecting women today. Due to the late onset of vague symptoms and a lack of reliable screening techniques, EOC is usually diagnosed in its advanced stages where the disease is very difficult to treat and the 5-year survival rate is low. Angiogenesis is the formation of new blood vessels from pre-existing vasculature and is believed to play an important role in tumor growth and metastasis of EOC. Thrombospondin-1 (TSP-1) is a potent anti-angiogenic extracellular matrix glycoprotein produced endogenously. We have previously shown that 3TSR, a peptide containing the three type-1 repeat regions of TSP-1, can normalize tumor vasculature, increase tumor perfusion and increase chemotherapy drug uptake in EOC solid tumors. Due to blood vessel normalization and increased tissue perfusion, we hypothesized that 3TSR would increase tissue uptake of chemotherapy drugs and enhance metronomic chemotherapy delivery to induce regression of advanced stage EOC. We treated our orthotopic, syngeneic mouse model with 3TSR alone and in combination with chemotherapy drugs administered with a maximum tolerated dose (MTD) and metronomic (MET) schedule. 3TSR combined with metronomic delivery of carboplatin and paclitaxel induced significant tumor regression. Combination of 3TSR with metronomic chemotherapy significantly decreased blood vessel density and increased survival compared to all other treatment groups. The results from this study showed that 3TSR in combination with metronomic chemotherapy had significant effects on tumor burden and this combination therapy may lead to improved clinical treatment for EOC in the future.

19) Treating high-grade glioma with oncolytic virotherapy and histone deacetylase inhibitors

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Primary brain cancer, specifically, high-grade glioma, in human patients has a median survival of 15 months. Despite aggressive treatment, virtually all tumours recur and are fatal. Novel therapies are required. Oncolytic virotherapy uses attenuated viruses that replicate in and kill cancerous but not normal cells. Recombinant vesicular stomatitis virus (rVSV) is a prototypical oncolytic rhabdovirus that is being developed for the clinical treatment of a variety of cancers. The selective replication of rVSV in malignant tissues is due to inherent defects in anti-viral type I interferon signaling, which is a common feature of many cancers. Some tumours maintain responsiveness to type I interferon but this can be blunted using histone deacetylase inhibitors (HDIs). This study evaluated the potential to use a recombinant oncolytic rhabdovirus in combination with histone deacetylase inhibition to kill primary brain cancer cell lines. We also tested the hypothesis that the replicating virus could be replaced with Toll-like receptor (TLR) ligands to potentiate the effect of a HDI. Initially, the oncolytic potential of rVSV was compared to a novel rhabdovirus known as recombinant maraba virus (rMG1). High-throughput therapeutic testing utilized a resazurin dye-based viability assay and the following glioma cell lines: murine GL261 and human U251, SF268, SF295, SNB19 and SNB75. The rMG1 proved to be a superior oncolytic virus in glioma cells. The HDIs entinostat, vorinostat, CI-994, tubastatin and PCI-34051 all proved efficacious as monotherapies, and were also tested in combination with rMG1. Notably, the data demonstrate an additive effect of rMG1 in combination with select HDIs, suggesting that this may represent a promising treatment for primary brain cancers. Current research aims to distinguish cytopathic versus cytostatic effects of the treatments by flow cytometric analyses and assessment of the responsiveness of the glioma cell lines to recombinant interferon beta.

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20) The Effects of 3TSR Fusion Proteins on Ovarian Cancer

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

Fc-3TSR has been demonstrated to potently reduce the invasiveness of ovarian tumour cells and regulates factors important for angiogenesis and tumor cell survival. While the ability of Fc-3TSR to exert more potent anti-angiogenic and apoptotic effects is still being investigated, our preliminary results suggest an increased efficacy of this compound when compared to its smaller counterpart 3TSR. We have shown that 3TSR can potently induce regression of advanced stage ovarian cancer in a mouse model of disease and the increased half-life of Fc-3TSR may induce regression even more dramatically.

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21) The development of recombinant *Parapoxvirus ovis* (ORFV) for use in oncolytic virotherapy.

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Oncolytic viruses (OVs) are anti-tumor agents that mediate the killing of cancer cells directly through infection or indirectly by disrupting tumor vascular architecture and/or activating the innate and adaptive arms of the immune system to modify the tumor microenvironment to be tumorcidal. OVs have several advantages over traditional therapeutic modalities, including the fact that they are highly tumor specific, they are self-amplifying and unlike other therapies, OVs can serve as both oncolytic agents and gene delivery vehicles. Parapoxvirus ovis or Orf virus (ORFV) is a member of the Poxviridae family, and is the etiological agent of orf, an acute and contagious dermatological disease affecting primarily ungulates, but also humans. ORFV is being investigated as a potential oncolytic virus due to its large genome, tropism for actively dividing cells, low to non-existent pre-existing immunity in the human population, and natural oncolytic properties. Indeed, live replicating ORFV has been shown to induce a vigorous antitumor immune response in at least two syngeneic mouse models of cancer through a mechanism that is largely mediated by the potent activation of both cytokine-secreting and tumoricidal natural killer (NK) cells. While initial results using wild type ORFV are very promising, the virus encodes a number of immune modulatory genes that, if removed, could further enhance the oncolytic and immune stimulating properties of the virus. Moreover, simultaneously engineering the virus to express transgenes that specifically stimulate the immune response against the tumor would serve to increase the efficacy of this novel therapeutic. Therefore the goal of this project is to capitalize on the unique biology of ORFV and through genetic manipulation of the virus, create an optimal OV platform for cancer treatment.

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22) Delivery of toxic topoisomerase mutant alleles by DNA ministrings for ovarian cancer gene therapy

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The use of conventional plasmids in gene therapy has been limited primarily by their large size and the presence of immunogenic prokaryotic genetic elements. DNA minivectors such as linearly covalently-closed (LCC) DNA minivectors and DNA minicircles are devoid of bacterial elements and show great promise as safe and highly efficient non-viral gene therapy vectors. We have previously developed enhanced LCC minivectors, or “DNA ministrings”, through a robust

and cost-effective one-step in vivo production platform comprised of a heat-inducible bacteriophage-derived recombination system in recombinant E. coli. DNA ministrings are highly efficient and safer than their plasmid or isogenic minicircle counterparts. Here we have employed DNA ministrings to encode toxic mutant topoisomerase alleles that mimic the action of topoisomerase poisons on DNA ministrings for delivery to targeted ovarian cancer cells as a novel gene therapy strategy. Topoisomerases are ubiquitous enzymes that regulate DNA topology through transient cleavage of the DNA helix. Topoisomerase poisons, such as camptothecin or etoposide, have long been used as effective anticancer agents and act by stabilizing the intermediate cleavage complex formed after a topoisomerase cleaves its DNA substrate. The stabilization of such intermediate nucleoprotein complexes leads to an accumulation of double-stranded DNA breaks, which in turn induces apoptosis or cell death. Specific negative dominant mutant topoisomerase alleles simulate the activity of such trapped intermediates in the absence of topo poisons. In this proof-of-principle study, DNA ministrings encoding the mutant topoisomerase alleles under the control of an ovarian tissue-specific promoter are transferred to ovarian carcinoma cells in vitro.
