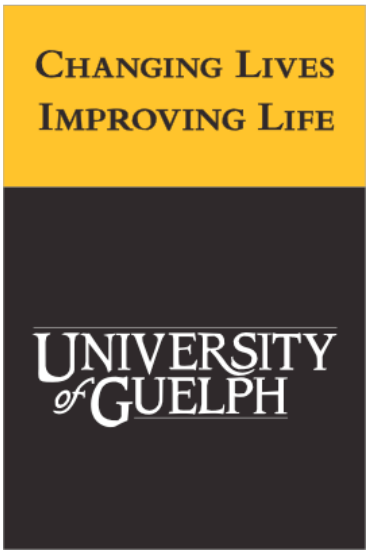
OVC | Institute for Comparative Cancer Investigation | University of Guelph

6th Annual Cancer Research Symposium Program

Thursday, May 23, 2013
Room 1714 LLC, Ontario Veterinary College
University of Guelph



Introductory Remarks

Welcome to the 6th 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. This year we are especially highlighting our existing and potential collaborations with 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. David Argyle, 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
Brenda Coomber and Tony Mutsaers
Biomedical Sciences and Clinical Studies, University of Guelph

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Thanks to Ms Barb Gaudette and Ms Carol Ann Higgins, OVC Office of the Dean, for their administrative expertise and invaluable assistance in organizing this event, 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: CIHR; NSERC; CFI; Government of Ontario; OMAFRA; OGS; Banting Research Foundation; Canadian Cancer Society Research Institute; Canadian Society for Immunology; GW Pharmaceuticals; Foundation for Research of Sao Paulo State (FAPESP), Brazil; OVC Pet Trust; Department of Biomedical Sciences, College of Biological Science, and the Ontario Veterinary College.

ICCI 6th Annual Cancer Research Symposium
Thursday May 23, 2013

Morning Session: Room 1714, OVC LLC

9:00 - 9:10 **Introductory remarks**

9:10 - 10:00 **Guest Speaker**
Jonathan Blay, School of Pharmacy, University of Waterloo, Waterloo, ON.
Department of Pathology, Dalhousie University, Halifax, NS
Two environments: The tissue microenvironment in colorectal cancer and the growing University of Waterloo environment in cancer research.

10:00 - 10:20 **Room 1707 B & C, OVC LLC**
Coffee Break

10:20 - 10:40
Nathan Farias, Department of Biomedical Sciences, University of Guelph
Colorectal cancer cells treated with varying levels of folic acid demonstrate altered DNA methylation and variable abilities to proliferate in suspension.

10:40 - 11:00
Roman Kondra, Molecular & Cellular Biology, University of Guelph
Role of the Wnt ligand in Nkd1's function and activation

11:00 - 11:20
Elizabeth Franks, Biomedical Science, University of Guelph
Divergent roles of Akt isoforms in insulin-like growth factor receptor-mediated lung tumorigenesis

11:20 - 11:40
Jim Petrik, Department of Biomedical Science, University of Guelph
The paradox of anti-angiogenic therapy

11:45 - 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

1:40 - 2:00
Jennifer Woo, Client Coordinator, Ontario Tumour Bank, Ontario Institute for Cancer Research
Ontario Tumour Bank - An international resource for cancer researchers

2:00 - 2:20
Courtney Schott, Pathobiology, University of Guelph
Design and construction of tissue microarrays for discovery of prognostic markers in canine cancers

2:20 - 2:40
Leanne Delaney, Department of Biomedical Sciences, University of Guelph
Involvement of Mcl-1 in the response of colorectal cancer cells to DCA treatment

2:40 - 3:00
Evan Lusty, Department of Pathobiology, University of Guelph
Characterizing oncolytic viruses, Toll Like Receptor ligands and histone deacetylase inhibitors in the in vitro treatment of human prostate cancer

3:00 - 3:20
Melissa Parsons-Doherty, Mona Campbell Centre for Animal Cancer, OVC HSC, University of Guelph
The efficacy and adverse event profile of Dexamethasone, Melphalan, Actinomycin D and Cytosine Arabinoside (DMAC) chemotherapy in relapsed canine lymphoma

3:20-3:30
Break

3:30 - 4:30
Keynote Speaker
Dr. David Argyle, Royal (Dick) School of Veterinary Studies and Roslin Institute, The University of Edinburgh
Title: A funny thing happened to me on the way to malignancy: Can comparative biology inform rational cancer drug development?

4:30 **Room 1707 B & C, OVC LLC**
Closing Reception

KEYNOTE PRESENTATION

3:30 OVC LLC Room 1714

Dr. David Argyle

BVMS PhD DECVIM-CA (Oncology) MRCVS, William Dick Professor of Veterinary Clinical Studies and Head, Royal (Dick) School of Veterinary Studies and Roslin Institute, The University of Edinburgh

A funny thing happened to me on the way to malignancy: Can comparative biology inform rational cancer drug development?

Despite an exponential growth in our understanding of cancer biology, for some cancers such as pancreas and gastric cancer, survival times have not improved in the last 100 years. Fundamental studies on tumor cell heterogeneity, cancer stem cells, metastasis programs and cellular targets have demonstrated significant promise in identifying therapeutic targets. Although several molecularly targeted drugs have entered clinical trials, failure to respond and the development of drug resistance remains a significant problem. In addition, we still rely on quite rigid linear drug testing pathways to move promise into clinical reality, a situation that is becoming unsustainable. In this lecture, we will explore how our changed understanding of classical cancer biology needs to inform drug development pathways. In this we will look at advances in comparative cancer and stem cell biology in dogs and cats, and how studies on naturally occurring malignancies in our domestic species may open up new clinical avenues in both human and veterinary oncology.

David Argyle graduated from the University of Glasgow. After a period in practice he returned to Glasgow to complete a PhD in Oncology/Immunology. He was senior lecturer in clinical oncology at Glasgow until 2002 when he became head of veterinary oncology at the University of Wisconsin-Madison, USA. In 2005 he returned to Edinburgh University to the William Dick Chair of Veterinary Clinical Studies. In 2009 he became the dean for postgraduate research and international for both medicine and veterinary medicine. In 2011 he was appointed as Head of School and Dean for the Royal (Dick) School of Veterinary Studies. He in an RCVS/European specialist in Veterinary Oncology, Diplomat of the European College of Internal Medicine in Oncology and co-scientific editor of the Journal of Veterinary and Comparative Oncology. His major research interests are cancer and stem cell biology.

Past Arthur Willis Distinguished Speakers
2012 Timothy Fan
2011 Cheryl London
2010 Matthew Breen
2009 Barbara Kitchell

GUEST SPEAKER

9:10 OVC LLC Room 1714

Dr. Jonathan Blay

Professor, Associate Director, Research and Graduate Studies, School of Pharmacy, University of Waterloo and Department of Pathology, Dalhousie University, Halifax, NS

Two environments: The tissue microenvironment in colorectal cancer and the growing University of Waterloo environment in cancer research.

The local environment of cells in colorectal cancer is a key determinant of both the expansion of the cancer and its ability to resist host defence and therapeutic attack. The influence of the environment is exerted through both the three-dimensional topology of the cancer cell population and its integration with other cell types, and soluble factors that act on the cancer cells from the extracellular fluid. Here, we consider those influences and how they impact both on our ability to elucidate the different mechanisms involved in colorectal cancer, and on how the results of research relate to the patient experience of cancer and our capacity to intervene in the course of neoplastic colorectal disease. Using the cell-surface protein CD26 as a readout of cellular behaviour we show how imposing an environment that authentically replicates the situation in vivo changes our perceptions of the behaviour of colorectal cancer cell behaviour and disease progression. We also describe methods that more accurately represent the disease in experimental models. Finally, we extend our viewpoint to the wider perspective of our research team itself, and comment on the transfer of our laboratory to a new location at the Pharmacy School in Waterloo, leading to new collaborations in the areas of medicinal chemistry, nutraceuticals and nanoparticle delivery that will allow greater exploration of novel interventions in colorectal and other cancers. We are forming a new cancer researcher network at the University of Waterloo and look forward to a great future relationship with our colleagues in ICCI.

Dr. Blay came to the Pharmacy School, University of Waterloo after being Professor of Pharmacology, Pathology and Biology at Dalhousie University, and Scientific Director of the Beatrice Hunter Cancer Research Institute in Atlantic Canada. A researcher with interests in many aspects of cancer therapy, his laboratory focuses on mechanisms that lead to the spread of colorectal cancer, and ways to interfere with that dissemination of disease, or metastasis. This research involves investigations of the cancer tissue microenvironment, chemokine pathways, and tumor-initiating cells, as well as the capacity of both synthetic and natural product-derived agents to interfere with the steps that favor metastasis. Dr. Blay is Associate Director of the School of Pharmacy has responsibility for both graduate studies and the expanding research in areas of pharmaceutical sciences, applied health sciences and pharmacy practice.

SHORT TALK ABSTRACTS: MORNING SESSION (*Study Leader)

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1) Colorectal cancer cells treated with varying levels of folic acid demonstrate altered DNA methylation and variable abilities to proliferate in suspension.

Nathan Farias, Brenda Coomber; Department of Biomedical Sciences, University of Guelph*

Mandated folic acid fortification, a B vitamin involved in DNA CpG methylation, has led to a subsequent increase in blood folate concentration as well as a simultaneous increase in colorectal cancer incidence in North America. My investigation looks at how low (0 mg/L), physiological (4 mg/L) and high (16 mg/L) levels of folic acid can influence cellular proliferation during cancer stem cell (CSC) cultivation. CSCs share properties with native stem cells and are vital in the development and perpetuation of tumor metastasis. Human colorectal cancer cell lines HCT116, Caco2, and SW480, LS174T, LIIA and PC7 (the latter two are sub lines of DLD-1), were cultivated in high, physiological and low folate conditions for seven days. Cell lines were then assessed for DNA methyltransferase1 protein expression, changes in DNA methylation and their ability to grow in CSC culture. CSC culture was used to promote the proliferation of CSCs and involves growing cells in suspension at low density and supplemented with FGF and EGF. Protein expression and DNA methylation were assessed using western blot and ELISA respectively. Aggressive colorectal cancer cell line models showed an increased ability to proliferate in CSC culture compared to less aggressive models (Caco2) and normal intestinal epithelium (IEC18). Low folic acid levels generally led to reduced DNA methylation and colonosphere yield while high folic acid levels led to increased DNA methylation and colonosphere yield. Thus, altered folic acid exposure *in vitro* can influence the methylation status and CSC self-renewal ability of human colorectal cancer cells.

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2) Role of the Wnt ligand in Nkd1's function and activation

Roman Kondra, Terry Van Raay; 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. Nkd1 is induced by Wnt signaling and seems to prevent β-catenin from accumulating in the nucleus. Nkd1 activity is dependent on plasma membrane localization via its myristoylation sequence, yet it functions in the cytoplasm to prevent nuclear accumulation of β-catenin. I hypothesize that in canonical Wnt signaling, Nkd1 activation is dependent on a Wnt ligand binding to its receptors, which transduces a signal to activate Nkd1. My project focuses on identifying Nkd1 modifications, such as phosphorylations, or interactions with other proteins. CRC's have mutations constitutively activating the Wnt pathway independent of a Wnt

ligand. Also, Nkd1 is upregulated in a large number of CRC's yet is insufficient to antagonize Wnt signaling. It is possible that without a Wnt stimulus, Nkd1 may be inactive and unable to abrogate Wnt signaling. Preliminary results show changes in Nkd1-GFP localization with and without Wnt stimulation in SW480 CRC cells. Currently, immunoprecipitations of Nkd1-Flag are underway to identify novel interacting proteins. 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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3) Divergent roles of Akt isoforms in insulin-like growth factor receptor-mediated lung tumorigenesis

S. Elizabeth Franks, Roger Moorehead; Biomedical Science, University of Guelph*

Akt is an important kinase in the PI3K pathway of growth factor signaling regulating cell growth, proliferation, survival, metabolism and migration. Increased Akt activation in lung cancer is associated with poor prognosis. Akt exists as three isoforms (Akt1-3) and while it was previously thought they were largely redundant in activity, evidence of differing roles is emerging. Our lab has generated a doxycycline-inducible, tissue-specific transgenic mouse model of lung cancer. Using a surfactant protein C (SPC) promoter, the type I insulin-like growth factor receptor (IGF-IR) is overexpressed in type II alveolar cells which initiates the development of tumors in the lungs. These lung adenomas typically present as one or more discrete nodules on the surface of the lung and express high levels of activated Akt. To investigate the role of specific Akt isoforms in lung tumorigenesis, these SPC-IGFIR transgenic mice were crossed with Akt1 null or Akt2 null mice to generate SPC-IGFIR-Akt1-/- and SPC-IGFIR-Akt2-/- mice. The Akt1 null SPC-IGFIR mice have reduced tumor burden compared to their Akt wild-type counterparts. In contrast, the absence of Akt2 accelerates tumor growth in SPC-IGFIR mice. While tumors formed in Akt1 null mice maintain the phenotype of distinct nodules, in Akt2 null mice the tumor tissue appears to permeate the lungs rather than form nodules. Initial results indicate opposing roles of Akt1 and Akt2 in the development and progression of lung cancer. While pan-Akt inhibitors are currently in clinical trials, our data suggests targeting Akt1 may be a more effective therapeutic strategy for lung cancer.

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4) The paradox of anti-angiogenic therapy

Jim Petrik, Department of Biomedical Science, University of Guelph

Solid tumors must recruit new blood vessels in order for sustained growth and metastatic dissemination. Often, however, tumor blood vessels are formed quickly, and are abnormal and dysfunctional, and tumor tissue hypoxia occurs. Most individuals receiving chemotherapy are treated systemically and rely on the vascular system to deliver drugs to the tumor, but because of dysfunctional vasculature very little drug reaches the tumor, limiting effectiveness and inducing chemoresistance. Anti-angiogenic drug therapy strives to inhibit the formation of tumor vasculature, thereby inhibiting tumor growth. However, while some studies show an anti-tumor

effect, several studies have demonstrated that tumor growth can accelerate during and after anti-angiogenic treatment. This paper will discuss results from our laboratory in which anti-angiogenic therapy with Thrombospondin-1 (TSP-1) results in tumor regression and reduction of abnormal tumor vasculature, with maintenance of healthy, mature vessels. Studies using our orthotopic, syngeneic mouse model of ovarian cancer, have shown increased tumor perfusion and enhanced chemotherapy drug uptake following TSP-1 therapy. The paradox of TSP-1 therapy lies in its ability to induce blood vessel regression, but only in abnormal, dysfunctional tumor vessels and actually results in increased vascular supply to the tumor. We believe we can exploit both sides of the paradox to significantly improve cancer therapy.

SHORT TALK ABSTRACTS: AFTERNOON SESSION (* study leader)

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1) Ontario Tumour Bank - An international resource for cancer researchers

Jennifer Woo, Dr. John Bartlett, Sugy Kodeeswaran*, Sally Stasi, Nancy Ahlan; Ontario Tumour Bank, Ontario Institute for Cancer Research*

Introduction/Background: The Ontario Tumour Bank (OTB) is a biorepository dedicated to the collection of human biospecimens and associated annotating data. OTB strives to provide a diverse selection of high quality tumour-related biospecimens, biospecimen derivatives and accompanying clinical data, to both academic and industry-based cancer researchers; to support the development of new diagnostic and prognostic tools, and the identification of targets for new drug development. OTB is a program of the Ontario Institute for Cancer Research (OICR). Funded by the Government of Ontario, OICR is a not-for-profit, innovative, translational research institute dedicated to research on the prevention, early detection, diagnosis and treatment of cancer.
Methodology: Standard operating procedures, tissue and data audits, quality analysis, as well as central and local governance ensure; donor consent, collection, processing, storage, and distribution of biospecimens, derivatives and data are in compliance with; international and Canadian standards of best practices, professional codes of conduct, institutional requirements and research ethics boards. Researchers are able to access fresh-frozen tumour and normal adjacent tissues, formalin fixed paraffin-embedded tissues, peripheral blood samples, DNA, RNA and tissue microarrays. Researchers worldwide can preview sample availability online and submit a sample request to gain access to this vital resource.
Results: As of March 2013, over 12 000 cases and 115 000 samples, along with comprehensive longitudinal clinical and pathological information, have been accrued. Biospecimens from over 35 different disease sites are available.
Conclusions: The OTB supports a wide spectrum of research, such as investigation to enable the discovery of: biological factors relating to susceptibility/risk, diagnosis, disease progression and response to treatment (i.e. biomarkers), targets for treatments, and host factors underlying the basis of drug reactions. The quality and integrity of biospecimens, derivatives and data directly affect the results of all succeeding scientific research.

2) Design and construction of tissue microarrays for discovery of prognostic markers in canine cancers

*Courtney Schott, Brian Stevens, Geoffrey Wood *; Department of Pathobiology, University of Guelph*

Tissue microarray (TMA) construction is a relatively new technology that provides a high throughput method for immunohistochemical investigations. Using TMA technology, histologic samples from several hundred individual tumours can be immunostained and evaluated on a single glass slide. This is accomplished by selecting small cores from different paraffin embedded tissue blocks and transferring the cores to holes in a single recipient block. The recipient block can then be sectioned on a standard microtome, yielding hundreds of tissue sections each containing hundreds of different tumour samples. This technique has several advantages over using full-tissue sections for immunohistochemistry. TMAs allow for: consistent sectioning, processing and staining across multiple tissue samples; savings on antibody costs and staining time; preservation of the original tissue blocks for other studies; as well as allowing examination of multiple tumours and control tissues almost simultaneously. The physical construction of a TMA is simple, but expertise in histopathology is necessary for the critical step of selecting representative tumour cores. Although TMA construction is a relatively lengthy process, the long term benefits are worth the investment as several hundred tissue sections can be made from a single TMA block. Further, this technique has particular advantages for veterinary studies as it allows for the incorporation of samples from multiple species, as well as multiple tumor types, into a single experiment. Application of TMA technology to canine mammary tumours and osteosarcomas for discovery of prognostic indicators and assessment of therapeutic target expression will be discussed.

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3) Involvement of Mcl-1 in the response of colorectal cancer cells to DCA treatment

Leanne Delaney, Brenda Coomber; Department of Biomedical Sciences, University of Guelph*

Recent evidence indicates that dichloroacetate (DCA) may be a cancer-specific agent that targets the unique metabolism of cancer cells, avoiding disruption of normal somatic cells. However, our laboratory has shown that some human colorectal cancer cell lines do not respond to DCA treatment as predicted, and the molecular mechanism responsible for this is currently unknown. Of interest is the Bcl-2 protein family, which consists of both pro- and anti-apoptotic members. The expression, regulation, and interactions of the Bcl-2 proteins will mediate apoptosis through mitochondrial outer membrane permeabilization. Differential regulation of the Bcl-2 proteins in response to DCA treatment may indicate whether apoptosis will be induced in cancer cell lines. Several human colorectal cancer cell lines were cultured and exposed to DCA or 5-fluorouracil, a known initiator of Bcl-2 protein-mediated apoptosis. Key changes in Bcl-2 protein expression were analyzed using immunoblotting. The expression of Mcl-1, an anti-apoptotic Bcl-2 protein, occurred at 40-kDa in all treatments, and at 28-kDa only in non-DCA treatments of HCT116 cells. PCR to detect known splice variants, investigation of binding partners, and manipulation of post-translational modifications to Mcl-1 allow further understanding of the changes in Mcl-1

associated with DCA treatment of HCT116 cells. Additionally, the role of Mcl-1 in DCA-induced changes in cell cycle is examined using immunofluorescence and flow cytometry. This research can provide insight into the mechanism by which some human colorectal cancer cells are able to evade DCA-induced apoptosis.

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4) Characterizing oncolytic viruses, Toll Like Receptor ligands and histone deacetylase inhibitors in the *in vitro* treatment of human prostate cancer

Evan Lusty, Byram W. Bridle; Department of Pathobiology, University of Guelph*

Oncolytic viruses (OVs) like Vesicular Stomatitis virus (VSV) and Maraba virus (MG1) represent novel cancer biotherapies with the potential to specifically replicate in and destroy tumours rather than non-cancerous tissues. While the oncolytic effect of the viruses is proving efficacious as a monotherapy there is interest in combining them with histone deacetylase inhibitors (HDIs). The HDIs promote histone protein acetylation and therefore, modulate gene expression. It has been demonstrated that this can potentiate the effects of OVs by dampening the type I interferon-mediated antiviral response in malignant cells, therefore potentiating the effects of the oncolytic viruses. However, we propose a hypothesis that reverses this paradigm. Instead of the HDIs promoting the oncolytic potential of OVs, we propose that OVs, or the immune response to the OVs, can potentiate the tumouricidal effects of the HDIs. Our data demonstrate that OVs in combination with HDIs can have additive effects. To prove that the direct oncolytic effects of the OVs are not required for this enhanced efficacy, irradiated (non-replicating) viruses or Toll-Like receptor (TLR) ligands that mimic infections are being used. The OVs VSV and MG1; HDIs suberoylanilide hydroxamic acid, CI-994, PCI 34051, SNDX-275 and Tubastatin A; and TLR ligands imiquimod, polyinosinic:polycytidylic acid and lipopolysaccharide were all tested as *in vitro* monotherapies against the PC3 human prostate cancer cell line. Viability measurements were made using a resazurin-based metabolic assay. Testing of the HDIs as monotherapies and in combination with OVs and TLR ligands is ongoing at this time.

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5) The efficacy and adverse event profile of Dexamethasone, Melphalan, Actinomycin D and Cytosine Arabinoside (DMAC) chemotherapy in relapsed canine lymphoma.

Melissa Parsons-Doherty, Valerie Poirier, Gabrielle Monteith; Animal Cancer Centre, Ontario Veterinary College Health Sciences Centre, Department of Clinical Studies, Ontario Veterinary College, University of Guelph*

In this retrospective study, the chemotherapy protocol using Dexamethasone, Melphalan, Actinomycin D, and Cytosine Arabinoside (DMAC) was evaluated for efficacy and adverse event profile in 86 client owned dogs with relapsed lymphoma. Forty-three (43%) achieved remission (16% complete remission [CR], 27% partial remission [PR]), and 57% were non–responders (NR). The median overall progression free survival (PFS) was 24 days. Adverse events included thrombocytopenia in 41% of dogs, neutopenia in 17% of dogs, and

gastrointestinal toxicity in 13% of dogs. Overall, 16% (13/79 dogs) experienced grade III to IV thrombocytopenia, 8% (6/74 dogs) grade III to IV neutropenia and 1% (1/79 dogs) grade III to IV gastrointestinal toxicity. These findings suggest that the efficacy of the DMAC protocol is not better than simpler rescue protocols when used in dogs with relapsed lymphoma and is associated with more toxicity.

POSTER ABSTRACTS (*study leader)

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1) The role of n-3 PUFA in breast cancer prevention through mammary stem cells and epigenetics: work in progress

Salma A. Abdelmagid, Lyn Hillyer, David W.L Ma; Department of Human Health and Nutritional Sciences, University of Guelph*

Growing evidence show that n-3 poly unsaturated fatty acids (PUFA) play a role in breast cancer (BC); however, research is still needed to substantiate their effect and to elucidate their mechanism of action. Recently we have shown that n-3 PUFA modify BC risk by breeding fat-1 transgenic mice, which synthesize n-3 PUFA from n-6 PUFA endogenously, with MMTV-neu(ndl)-YD5 mice, which is an aggressive BC model. The hybrid progeny had a 30% reduction in tumor volume and multiplicity. Building on these findings, and based on the notions that BC is a genetic and epigenetic disease and that mammary stem cells (MaSC) may serve as target of oncogenic changes, we sought to determine the role of n-3 PUFA in the mammary epithelial cell (MEC) profile. Fat-1 and wild type (WT) mice were maintained on diet containing 10 % safflower oil. At 6 weeks of age mice were terminated and MEC were isolated. Surface expression of CD24 was assessed by flow cytometry to determine specific MEC populations. Fat-1 mice expressed 65% non-epithelial, 20% myoepithelial and 15% luminal epithelial while WT mice expressed 65%, 26% and 9% for non, myo and luminal epithelial cells, respectively. The luminal epithelial population, which exhibits stem cell characteristics, was greater in the fat-1 mouse (P=0.005). Results suggest that n-3 PUFA alters the proportion of MEC in the developing mammary gland, which may have implications for cancer risk. Future experiments will compare gene expression and epigenetics.

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2) Targeting Heat Shock Protein 70 inhibition for treatment of canine osteosarcoma

Jonathan Asling, Anthony Mutsaers; Department of Biomedical Sciences, University of Guelph*

Osteosarcoma (OSA) is the most common form of primary bone cancer in both humans and canines. These tumours are highly aggressive and metastasize readily. Despite the improved survival seen with the use of surgery and adjuvant chemotherapy, further advances in medical treatment are required to effectively slow metastatic disease and prolong patient survival. Heat shock proteins (HSPs) are considered some of the most highly conserved proteins in eukaryotes. These proteins function as molecular chaperones under normal physiological conditions. However, HSP production is rapidly induced by various forms of cellular stress such as hypoxia, nutrient deprivation and anticancer treatment. Under these conditions the cytoprotective role of heat shock proteins may prevent apoptosis. One such HSP family member, HSP70, has been identified as a marker for malignancy and prognosis in various forms of cancer, including OSA. Treatment with VER155008, an N-terminal ATPase inhibitor of HSP70, inhibited proliferation/viability of canine OSA cell lines when used as a single agent, or given in

combination with the chemotherapy agent doxorubicin. However, in response to HSP72 inhibitor treatment, canine OSA cells up-regulate HSP70 itself and another HSP70 family member: GRP78. GRP78, which is normally found within the endoplasmic reticulum, was subsequently dispersed throughout the cytoplasm and translocated to the nucleus. Lower dose, metronomic scheduling of doxorubicin revealed that treatment with VER155008 was slightly more effective when administered prior to doxorubicin. Given the upregulation of HSP70 family members and their potential collective effect on apoptosis, further studies are warranted to optimize HSP inhibition for targeted cancer therapy.

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3) Analysis of the phosphotyrosine adaptor protein ShcD in developmental and tumourigenic signal transduction

Jim Cooper; Dr. Nina Jones; Department of Molecular and Cellular Biology, University of Guelph*

The regulation of biological processes is contingent on appropriate cell signaling events maintained by stringent protein-protein interactions. Understanding the molecular mechanisms by which these interactions occur is paramount to furthering our collective knowledge in developmental biology and human disease. The Src homology and collagen (Shc) family of phosphotyrosine adaptor proteins are intricate facilitators of both essential and aberrant signaling events. Concurrently, evidence from our laboratory and others implicate ShcD expression, the most recent addition to the Shc family, in numerous cell types including immature oligodendrocytes and metastatic melanoma. This project has focused on the characterization of the ShcD signaling molecule to better understand its role in developmental and tumourigenic signal transduction.

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4) Potential of Canine & Feline Pancreatic Carcinoma as a Model for Human Pancreatic Carcinoma

Cheryl Crozier[1], Brenda Coomber[2], Robert Foster[3], Sally Stasi[1], John M.S. Bartlett[1], Vicky S. Sabine[1] [1] Ontario Institute for Cancer Research, Toronto; [2]Biomedical Sciences and [3]Pathobiology, Ontario Veterinary College, University of Guelph*

The poor survival rate of human pancreatic carcinomas (6% over 5-yrs) highlights the need for improving current treatment. Companion animals, which develop cancers spontaneously and share many of the same genetic features as humans, may serve as valuable models for studying this disease. KRAS is the most commonly mutated gene in human pancreatic cancers (57%), with 24 different mutations reported across 7 codons, occurring most frequently at codons 12, 13, and 61. Codon 12 is the most frequently mutated site accounting for 98% of KRAS mutations, of which G12D (49%), G12V (29%), G12R (12%), G12C (3%), and G12A (2%) are the most common. Mutations have also been found to occur in codons 13 (1%) and 61 (1%), primarily G13D (0.83%), and Q61H (0.63%), respectively. In this study 32 pancreatic tumour samples were obtained from 14 dogs and 18 cats including 6 metastases. Mutational analyses

were performed on 6 KRAS mutations located in codons 12 (G12D, G12V, G12R, G12C G12A), and 13 (G13D) using Sequenom MassARRAY. No KRAS mutations were found. Although sample size was limited, the results suggest that KRAS mutations do not play a critical role in feline or canine pancreatic carcinomas and that companion animals may not provide a suitable model for studying human pancreatic carcinoma from a KRAS mutation perspective.

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5) Role of Different Transforming Growth Factor Beta (TGFβ) Isoforms in Angiogenesis

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

Angiogenesis is the formation of new blood vessels from pre-existing ones via endothelial cell (EC) sprouting, and it's required for vascular development, adult tissue homeostasis, tumor growth and metastasis. This process is regulated by signaling crosstalk of multiple growth factor-receptor systems. Of particular interest is the crosstalk between vascular endothelial growth factor (VEGF) and transforming growth factor-beta (TGFβ) signaling. Low concentrations of TGFβ1 (up to 0.5 ng/mL) were previously shown to cooperate with VEGF in stimulating an angiogenic response in cultured ECs. However, it is not clear whether this holds true for all TGFβ isoforms. TGFβ isoforms 1 and 2 have been shown to have different, non-over lapping roles in vascular development and cancer. Therefore, we hypothesize TGFβ's cooperation with VEGF to drive the angiogenic response is modulated by the type of TGFβ isoform present. Our first objective to test this hypothesis is to evaluate canonical (mediated by Smad2/3) TGFb signaling activation and angiogenic response of bovine aortic endothelial cells (BAEC) and human pulmonary endothelial cells (HPMEC) to TGFβ1 and TGFβ2 in the presence or absence of VEGF. Preliminary western blot and immunofluorescence analyses demonstrate that TGFβ1 treatment activates TGFβ signaling in BAECs, indicating cellular response to TGFβ1 in this cell line. In addition both cell lines formed tube-like structures in response to TGFβ1 and VEGF in Matrigel™ and collagen type IV. This research is important to improve our understanding of TGFβ's role in tumor angiogenesis and can potentially contribute to the development of more effective anti-angiogenic cancer therapies.

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6) Effect of Melatonin in apoptosis of immortalized and transformed murine mammary cells

Gabriela Bottaro Gelaleti[1], Debora Ap. Pires de Campos Zuccari[1], Alicia Viloria-Petit[2];*
[1]Department of Biology, Paulista State University - UNESP/IBILCE, Brazil; [2]Department of Biomedical Sciences, University of Guelph

Mammary carcinoma is the most common cancer among women and the fifth leading cause of cancer-related death. It is also frequent in dogs, where most therapeutic strategies are not effective at preventing tumor relapse. Melatonin is a natural hormone produced and secreted in the pineal gland, which has shown promising results as an anti-tumor agent in both in vitro and in vivo models of mammary cancer. This is in part due to its capacity to promote apoptosis of

cancer cells by mechanisms that are not fully understood. As a first step to investigate melatonin's pro-apoptotic function in mammary cancer we compared the effects of this hormone on immortalized versus transformed murine mammary cells NMuMG and EMT-6, respectively. Sub-confluent cell monolayers were exposed to increasing concentrations of melatonin for 24 hours and the expression of caspase 3 and cleaved caspase-3 (a marker of apoptosis) was evaluated by western blotting. Triplicate experiments were performed. Melatonin caused a dose-dependent increase in cleaved caspase 3 only in EMT-6 cells, where a significant 5-fold increase was observed at the 1 mM concentration. These results confirm the previously reported capacity of melatonin to induce apoptosis in transformed cells. We are currently evaluating melatonin's effect on apoptosis of canine mammary cell lines, as our ultimate goal is to assess the possibility of using this cost-effective hormone for the treatment of canine mammary cancer.

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7) The Par6 pathway modulates apoptosis, cell survival signaling and key mediators of polarity disruption in response to TGFβ.

Richard W.D. Gilbert[&], Geordon Avery-Cooper[&], Mahmoud Youssef, Alicia Viloria-Petit; Biomedical Sciences, University of Guelph; [&]Authors contributed equally.*

Transforming growth factor beta (TGFβ) plays paradoxical roles during breast cancer progression, where it is known to act both as a tumor suppressor and as a promoter of invasiveness and metastasis. The tumor suppressor function of TGFβ is driven by its capacity to inhibit cell growth and induce apoptosis, while its pro-invasiveness function has been associated to its ability to induce epithelial-mesenchymal transition (EMT). Using immortalized (pre-malignant) and transformed mouse mammary cells grown as three-dimensional acini-like structures we have previously shown that the non-canonical Par6 pathway, which mediates the dissolution of tight junctions and rearrangement of the actin cytoskeleton in response to TGFβ, is essential for both the pro-apoptotic and EMT inducing functions of TGFβ. Here we expand these observations by providing evidence that TGFβ's pro-apoptotic function is closely linked to its capacity to promote full loss of apico-basal polarity in pre-malignant cells, and that both of these processes (loss of polarity and apoptosis) require cooperation between the Par6 and canonical Smad pathways. We have also observed that, exposing Par6 overexpressing pre-malignant mammary cells to TGFβ treatment significantly potentiates the apoptosis response to TGFβ, and this phenomenon is dependent on Par6 signaling. Further, Par6 signaling appears to modulate apoptosis in a manner that differs from canonical Smad signaling, in that it inhibits survival pathways as compared to the induction of apoptosis mediators characteristic of Smad activation. We propose the modulation of polarity-associated survival signaling networks as a novel approach to induce cell death in mammary tumors.

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8) Evaluating the role of Nck adaptor protein signalling in cardiovascular development and EnMT

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Endothelial to mesenchymal transition (EnMT) is a tightly regulated process required for proper embryonic development. EnMT involves the cell changing its morphology from a polarized endothelial cell to a motile mesenchymal cell. It is naturally occurring in the endocardial cushion formation of the embryonic heart, but is also associated with various pathological conditions, including tumour progression. Our lab has previously demonstrated that loss of expression of Nck1 and Nck2 adaptor proteins in endothelial cells leads to altered cardiovascular development, with disrupted endocardial cushion formation in vivo and reduced cell growth from ex vivo heart explants. We this hypothesize that Nck may participate in a signalling pathway that regulates aspects of EnMT. Using a genetically modified mouse model, in vivo immunofluorescence complimented by in vitro analysis of EnMT markers, a role for Nck in EnMT should be validated while beginning to elucidate potential signalling pathways. Should our hypothesis hold true, we expect to see sustained expression of endothelial markers and limited mesenchymal markers in Nck null mutants. Determining what role Nck plays in EnMT could provide new insights into signalling pathways involved in developmental EnMT that could extend into pathological conditions, highlighted by tumour progression.

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9) Molecular considerations when using dichloroacetate to reverse the Warburg effect in human colorectal cancer cells

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Dichloroacetate (DCA) is a small molecule metabolic modulator that shifts cellular metabolism to glucose oxidation by inhibiting pyruvate dehydrogenase (PDH) kinase (PDK), thereby reversing the Warburg effect commonly exhibited by cancer cells. DCA can induce apoptosis in cancer but not normal cells. Our previous work showed that some human colorectal cancer (CRC) cells are protected from anoxia-induced apoptosis upon exposure to DCA, through as yet unclear mechanisms. It is hypothesized that differential expression of PDK isoforms will correlate with the ability of different cancer cells to withstand the apoptotic effects of DCA in anoxia. Human CRC cell lines were exposed to 10 mM DCA in anoxia for 24 hours. Following treatment, expression of PDK isoforms was determined through qRT-PCR and western blotting. PDH activation was assessed through western blotting. Metabolic changes were assessed via mitochondrial activity, glucose consumption and lactate production. Cell viability was examined using the neutral red assay. We found that PDK isoforms 1 and 3 are highly expressed relative to 2 and 4 in our cell lines, and that DCA alone did not alter PDK expression. Effects of DCA and/or anoxic treatment on PDH phosphorylation at site S293 was cell-dependent. DCA had minimal effect on metabolic outputs and cell viability. Altered PDK expression and PDH activation may not fully explain differences in the resistance to DCA-induced apoptosis previously observed in these cells. Investigating the mechanisms by which this cytoprotection may occur will lead to a better understanding of cancer metabolism and thus provide better therapeutic opportunities.

10) The Wnt negative feedback regulator Nkd1 is activated by Wnt ligand to antagonize the nuclear accumulation of β-catenin

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Wnt signaling is a highly conserved pathway in both invertebrates and vertebrates with an important role in early embryogenesis and maintenance of stem cells in adult tissue. It is now known that Wnt signaling also plays a critically important role in the initiation of cancer. Therefore, fully elucidating the signaling events of this pathway will have important consequences in understanding how misregulation of this pathway contributes to disease. One such event is the induction of the negative feedback regulator, Nkd1, which antagonizes Wnt signaling in the cytoplasm by inhibiting the nuclear accumulation of the central signaling component β−catenin. Curiously, Nkd1 is required to associate with the plasma membrane for proper function. Therefore, my research tests the hypothesis that Nkd1 is activated upon Wnt ligand interacting with its receptor to then antagonize nuclear accumulation of β−catenin. Using Wnt target gene analysis in the early zebrafish embryo, we tested the ability of Nkd1 to antagonize a Wnt Ligand versus downstream agonists. We found that while Nkd1 could effectively antagonize Wnt ligand, it could not antagonize the downstream agonists, unless we added excess Wnt ligand. Visualizing the localization of β−catenin with Wnt ligand or downstream agonists further substantiated this. Furthermore, we observe a redistribution of Nkd1 From the membrane to become more cytoplasmic in the presence of Wnt ligand. Taken together this data demonstrates that Nkd1 requires the presence of a Wnt ligand interacting with its receptors to then move into the cytoplasm to interact with β−catenin and prevents its nuclear accumulation.

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11) Effect of Metformin on TGF-beta induced epithelial mesenchymal transition in mammary cells

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The epithelial–mesenchymal transition (EMT) is defined by the loss of epithelial characteristics and the acquisition of a mesenchymal phenotype, and has been shown to drive aberrant cell–cell and cell-matrix interactions that facilitate tumor dissemination. EMT is induced by extracellular matrix components and growth factors. Transforming growth factor-beta (TGFβ), commonly overexpressed in breast cancer, is one of the best-documented inducers of EMT. The drug metformin, on the other hand, has been reported to inhibit EMT by suppressing the expression of EMT transcription factors in breast cancer cells. Metformin is a hypoglycemic agent used to treat diabetes, and the use of this drug has been associated with lower incidence of breast cancer. Metformin also inhibits growth, invasion and metastasis of breast cancer cell lines. Based on this evidence we hypothesize that metformin antagonizes the EMT promoting effects of TGFβ. To address this, we treated immortalized (NMuMG) and transformed (CF-41) mammary cells with TGFβ1 in the presence or absence of 1 mM or 10 mM metformin for 24 hours. We used western

blotting to examine the expression of the EMT markers E-cadherin and N-cadherin, which decrease and increase, respectively, in response to TGFβ. 10 mM metformin suppresses the induction of N-cadherin by TGFβ. Since both TGFβ and N-cadherin have been shown to promote metastasis, these results suggest that negative modulation of TGFβ-induced N-cadherin expression may contribute to the anti-metastatic properties of metformin. Experiments including additional EMT markers and cell lines, as well as longer treatment times are underway to confirm these preliminary findings.

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12) Attenuation of anticipatory nausea in a rat model of contextually elicited conditioned gaping by manipulation of the endocannabinoid system

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Following chemotherapy treatments, many patients report experiencing anticipatory nausea (AN), where a conditional association developed between the contextual clinic cues and post-treatment nausea. Once AN develops it is resistant to anti-nausea treatments. Rats express conditioned gaping reactions when exposed to a context previously paired with an emetic agent, a response that serves as a rodent model of nausea-like behavior. We evaluated the potential of the dual FAAH/MAGL inhibitor, JZL195 on its own and combined with anandamide (AEA) or 2-arachidonoyl glycerol (2-AG) to reduce conditioned gaping. Over 4 conditioning trials rats were injected with Lithium Chloride and placed in a distinctive context for 30 min. For the 5 min test of AN, rats were pretreated with vehicle or JZL195 prior to placement in the nausea-paired context. Additional groups were injected with AEA or 2-AG prior to placement in the chamber. Finally, the potential of the CB1 antagonist/inverse agonist SR141716 to reverse suppression of AN was evaluated. Brains were removed for tissue analysis of AEA and 2-AG levels. JZL195 suppressed conditioned gaping, an effect that was reversed by SR141716. Combined pretreatment with AEA or 2-AG amplified the suppressive effect of JZL195 alone. AEA pretreatment suppressed conditioned gaping, an effect also reversed by SR141716. Whole brain analysis revealed a JZL-induced increase in AEA but not 2-AG levels, which was dramatically potentiated by exogenous AEA. Manipulations of the endocannabinoid system may have therapeutic potential in the treatment of AN.

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13) Genome-wide comparison of matched canine osteosarcoma primary tumours and metastases by array comparative genomic hybridization

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Canine osteosarcoma is a common and highly aggressive cancer with a particularly grave prognosis. We collected normal muscle, primary appendicular skeleton tumour, and matched terminal lung metastases from 8 canine osteosarcoma patients. Gene copy number aberrations were assessed using high-resolution oligonucleotide array comparative genomic hybridization (Agilent G3 Canine 180k array). To assess the degree of genomic similarity of the primary tumours to their corresponding metastasis, we generated 8 random pairings of unmatched primaries and metastases, as well as 8 random pairs of primary tumours and 8 random pairs of metastases. A correlation analysis was conducted for each pair using the $\log_2$ ratios for each of 11,000 random 10 base pair segments spanning the entire genome. Matched samples showed significant correlation with the square of the correlation coefficient (R^2) being 0.44 ($p<0.05$), whereas unmatched primaries and metastases, random pairs of primaries, and random pairs of metastases did not show significant correlation (R^2= 0.08, 0.08, and 0.06 respectively, $p>0.05$). Thus, as a group, primary tumours are genomically more similar to their corresponding metastasis than to unmatched metastases, and are also more similar than primary tumours or metastases across cases. However, some primary tumours did not match well to their corresponding metastases. This has implications for therapies that rely on analysis of primary tumour samples for targeted therapy, since targeted genes may be unaltered or even deleted in metastases.

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14) The role of TAZ in the induction of metastasis-associated traits in canine osteosarcoma

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Osteosarcoma (OSA) is the most common primary bone tumor in humans and dogs and often presents with metastasis at diagnosis. Metastatic OSA is resistant to current treatment modalities and is ultimately responsible for OSA-associated mortality. Understanding what drives metastasis of OSA is necessary to identify new therapeutic targets. We propose that TAZ, a transcriptional co-activator involved in bone development and in cellular response to transforming growth factor-beta (TGFβ), could be this potential target. We have previously observed that TAZ is overexpressed in metastatic human OSA cell-lines as compared to non-metastatic ones, and suppressing TAZ expression inhibits TGFb response in the metastatic cells. Given that both TAZ activity and TGFβ signaling has been shown to promote metastasis, stemness and therapy resistance, *we hypothesize that high TAZ expression promotes the highly metastatic and therapy-resistant behavior of OSA.* Thus, we performed Western blotting and immunofluorescence studies on a panel of canine OSA cell-lines to (i) characterize expression and localization of TAZ in response to TGFβ1 and, (ii) characterize the expression of TGFb1-induced transcription factors (Snail1, Twist1) known to promote metastasis, stemness and tumor

recurrence. We found variable basal levels of TAZ in the cell-lines, but a consistent dose-depended induction of TAZ expression after 24 hours of TGFβ1 treatment (p<0.005; n=3), which paralleled a dose-dependent induction of Snail1. TGFb1 treatment (5 ng/mL) also promoted TAZ translocation with activated Smad2 (a marker of TGFb signaling) at the cell nucleus. Taken together, these results support the potential involvement of TAZ in OSA morbidity.

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15) Inhibiting anandamine transport: The impact of prolonged anandamine availability on nausea

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Considerable evidence supports anandamide (AEA) as an important mediator in the regulation of nausea. Recently reported was a protein that mediates the cellular transport of AEA, FLAT (FAAH-1-like AEA transporter). Here we present evidence that inhibiting FLAT activity with ARN272 (ARN) produces an indirect agonism of CB1 receptors to attenuate nausea and vomiting. We used a Lithium Chloride (LiCl)-induced conditioned gaping model of nausea in rats. In Experiment 1a, rats were injected with the AEA transport inhibitor, ARN (0.1, 1.0, or 3.0 mg/kg, i.p.) or VEH, 2 hr prior to behavioral conditioning. Two conditioning sessions separated by 72 hours were followed by a drug free test day. Experiment 1b, evaluated the potential of the CB1 antagonist SR141716 (1.0, 2.5 mg/kg) given 30 min prior to conditioning, to reverse the ARN suppressed gaping. In Experiment 2, ARN's ability to interfere with LiCl-induced vomiting in the *Suncus murinus* (house musk shrew) was investigated. Administration of ARN produced a dose-dependent reduction of LiCl-induced conditioned gaping, with 3 mg/kg being the optimally effective dose. The suppression of conditioned gaping by ARN was reversed partially by SR at 2.5 mg/kg, and fully at 1.0 mg/kg. The reversal of ARN's effects by SR suggests a CB1 receptor mechanism of action. ARN also attenuated LiCl-induced vomiting in the house musk shrew at 18 mg/kg. The results suggest that preventing the cellular reuptake of AEA through transport inhibition tonically activates CB1 receptors to regulate toxin-induced nausea.

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16) Cannabidiolic acid and tetrahydrocannabinolic acid reduce conditioned gaping (nausea-induced behaviour) in rats and vomiting in Suncus murinus

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The phytocannabinoids Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD) reduce nausea and vomiting (NV), however less is known about their acidic precursors. Previous research indicates that cannabidiolic acid (CBDA), CBD's precursor, potently reduces vomiting in *Suncus murinus* and conditioned gaping in rats to a lithium chloride (LiCl)-paired flavour (measure of

nausea-induced behavior) and to a LiCl-paired context (a rodent model of anticipatory nausea); effects mediated by the 5-hydroxytryptamine 1A (5-HT1A) receptor (Bolognini et al., 2013). The present studies assessed the minimally effective CBDA dose to reduce conditioned gaping and whether a subthreshold CBDA dose could enhance the anti-nausea effects of a low dose of ondansetron (OND, a 5-HT3 receptor antagonist). The anti-emetic/anti-nausea properties of tetrahydrocannabinolic acid (THCA), THC's precursor, were assessed in these models to determine its mechanism of action. CBDA (0.5 μg/kg) suppressed conditioned gaping. A low dose of OND (1.0 μg/kg) reduced conditioned gaping, but when combined with a subthreshold CBDA dose (0.1 μg/kg), enhanced suppression of conditioned gaping. These findings suggest combining low doses of CBDA and OND could effectively manage acute nausea. THCA (0.05 and/or 0.5 mg/kg) suppressed conditioned gaping to a flavour and to a context; an effect blocked by the CB1 receptor antagonist, SR141617 (SR). In S. murinus, THCA (0.05 and 0.5 mg/kg) reduced vomiting; an effect reversed by SR. These data suggest that THCA may be a potent non-psychoactive alternative to THC in managing NV.

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17) 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 effecting 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 five-year survival rate is approximately 20%. Angiogenesis is the formation of new blood vessels from pre-existing vasculature and is believed to play an important role in the growth and metastasis of EOC. Thrombospondin-1 (TSP-1) is a potent antiangiogenic 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 hypothesize that 3TSR will increase tissue uptake of chemotherapy drugs and synergize with metronomic chemotherapy delivery to induce regression of advanced stage disease. 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 chemotherapy drug treatment increased survival compared to untreated mice, or those treated with the drugs individually. The results from this study suggest that 3TSR in combination with metronomic chemotherapy has a significant effect on tumor burden and may be a possible clinical treatment for EOC in the future.

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18) Tissue Microarray Design - An intentional approach

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A tissue microarray (TMA) is a "recipient" paraffin block that contains tissue cores from different "donor" formalin fixed paraffin embedded (FFPE) tissue blocks, in a defined layout. TMA blocks facilitate the simultaneous analysis of hundreds of tissue specimens on a single microscope slide. This reduces reagent and labour costs and allows for uniform analysis of all the specimens included in the TMA. In addition, TMAs maximize the use of valuable tissue sources as the tissue cores range from 0.6mm-1mm in diameter, leaving much of the "donor" block available for future use. The field of histology is still one where automation and ready-to-use kits have not eliminated the need for trained personnel and skilled hands. While automated arrayers exist to facilitate the construction of TMAs, the selection of areas of interest (ex. tumour marking) and the design of the TMA still require persons qualified, by education and training, in histology.

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19) Experimental transmission of enzootic nasal adenocarcinoma in sheep

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Enzootic nasal adenocarcinoma (ENA) is a contagious neoplasm of the secretory epithelial cells of the nasal mucosa of sheep and goats. It is associated with the betaretrovirus, enzootic nasal tumor virus (ENTV), but a causative relationship has yet to be demonstrated. In this report, 14-day-old lambs were experimentally infected with cell-free tumor filtrates derived from naturally occurring cases of ENA via nebulization. At 12 weeks post-infection, one of the five infected lambs developed clinical signs, including continuous nasal discharge and open mouth breathing, and was euthanized. Necropsy revealed the presence of a large, space occupying bilateral nasal tumor. At 45 weeks post-infection, when the study was terminated, none of the remaining sheep showed evidence of tumors either by computed tomography or post-mortem analysis. Examination of the tissue distribution of the ENTV provirus in experimentally and naturally infected animals revealed a similar pattern, thereby validating the infection method used in this study. The tumor phenotype of the experimentally induced ENA differed from that of naturally occurring ENA appearing more malignant in nature – a feature that might be attributed to the rapid growth of the tumor. A significant proportion of virus particles purified from the experimentally induced nasal tumor had a buoyant density of 1.22g/ml, higher than that of virus particles from naturally induced ENA, and this corresponded with a greater proportion of immature unprocessed virus particles. Despite the apparent lack of processing, mature virus particles with a similar morphology as was seen in naturally occurring ENA could be identified by electron microscopy.

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20) Effects of sequential combination chemotherapy on the fecal microbiome in dogs with lymphoma

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Recent advances in molecular technologies have allowed for comprehensive evaluation of the intestinal bacterial population (microbiome). The objective of this study was to characterize the fecal microbiome of dogs with lymphoma by high throughput sequencing technology, and to investigate the impact of chemotherapy. Fecal samples were collected at initial presentation and at week 9 from 4 dogs with multicentric lymphoma treated with a 25 week CHOP protocol. DNA was extracted and PCR of the 16S rRNA gene was performed. Amplicons were sequenced by high throughput sequencing technology. The MOTHUR package of algorithms was used to clean data and align sequences with the SILVA 16S rRNA reference database, with taxonomic classifications obtained from the Ribosomal Database Project. Sequences were assigned into operational taxonomic units (OTUs) using the average neighbor algorithm. 25,630 high quality sequences remained after data cleaning. For OTU classification, a minimum of 1,511 sequences per dog was used. Eight phyla were identified. Firmicutes was the main Phylum among dogs before and after 9 weeks of chemotherapy (55.7 and 51.2%, respectively). There was no statistical difference in the abundance of any of the major phyla between groups (all P=0.1). On phylogenetic tree, data were clustered by individuals and not by treatment, indicating the inter-dog variation was greater than the effect of treatment. No apparent changes in the fecal microbiome were evident after 9 weeks of chemotherapy. Gastrointestinal effects of chemotherapy might be more related to changes in immune status or other factors rather than alteration of the gut microbiome.