OVC | Institute for Comparative Cancer Investigation | University of Guelph

Program

Cancer Research Symposium

Wednesday, May 16, 2012
Room 1714 LLC, Ontario Veterinary College
University of Guelph

Introductory Remarks

Welcome to the 5th 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's particular themes includes an exploration of the variety of cancer models being used on campus to improve our understanding. 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. Timothy Fan, 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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Additional thanks to Ms Barb Gaudette, OVC Office of the Dean, for her administrative expertise and invaluable assistance in organizing this event, to University of Guelph Hospitality Services and Physical Resources, and to the 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, Agriculture and Agrifoods Canada, Ontario MRI, OMAFRA, OGS, CIBC, Canadian Cancer Society, Crohn's and Colitis Foundation of Canada, VSSO, National Natural Science Foundation of China, GlaxoSmithKline, University of Guelph GFRC, OVC Pet Trust, Department of Biomedical Sciences and the Ontario Veterinary College.

ICCI 4th Annual Cancer Research Symposium
Wednesday May 16, 2012, Room 1714 OVC LLC

Room 1714 OVC LLC

9:00 Welcome and introduction

Theme 1: Modelling Cancer In Vivo, In Vitro and In Silico

9:10-9:35 Tony Mutsaers, Biomedical Sciences and Clinical Studies, OVC
"Modeling distinct osteosarcoma subtypes in vivo using lineage restricted transgenic shRNA"

9:35-9:50 *Short talk:* Terry Van Raay, Molecular and Cellular Biology "*Nkd1 is activated by Wnt to prevent nuclear accumulation of β-catenin*"

9:50-10:15 Chris Bauch, Mathematics and Statistics, CPES
"Time for change? An economic evaluation of cervical cancer prevention in Canada"

10:15 - 10:35 ***Coffee Break Room 1707 B & C***

10:35-11:00 Byram Bridle, Pathobiology, OVC
"*Paradoxically, immunotherapy might be more efficacious when tumours are inside the brain*"

11:00-11:15 *Short talk:* David K.Y. Chiu, School of Computer Science "*Evaluating sites of post-translational modifications in p53 using Convergent Association Testing*"

11:15-12:45 Lunch (provided) and Poster Session, Room 1707 B&C.
Poster presenters please attend your posters between 11:30 and 12:30

Room 1714 OVC LLC

Theme 2: Nutritional Insights and Impacts

12:45-1:10 Emma Allen-Vercoe, Molecular and Cellular Biology, CBS
" *Fusobacterium nucleatum*: an emerging gut pathogen associated with colorectal cancer**"**

1:10-1:25 *Short talk*: Leila Zarepoor, Human Health & Nutritional Sciences, CBS
"*Effects of flaxseed and its seed components on ulcerative colitis in female mice*"

1:25-1:40 *Short talk:* Dasha Alimenkova, Human Health & Nutritional Sciences, CBS
"*The effect of nicotinamide supplementation on caspase-independent cell death in HeLa cervical carcinoma cells*"

1:40-2:05 *Short talk:* Kate Perez, Human Health & Nutritional Sciences, CBS
"*Omega-3 fatty acids reduce mammary gland tumour development in MMTV-neuYD5 mice*"

Theme 3: The Patient and Their Disease

2:05-2:30 Sarah Boston, Clinical Studies, OVC
"*Palliative treatment of osteosarcoma in dogs: what should we do*?"

2:30-2:45 *Short talk:* Cathy Furness, Clinical Studies, OVC
"*Basophilic leukemia in a standardbred gelding*"

2:45-3:00 *Short talk:* Debbie Stoewen, Population Medicine, OVC
"Factors associated with the recommendation of a referral to specialty oncology services"

3:00- 3:15 Break

3:15 Keynote Address Room 1714 OVC LLC

Dr. Timothy M. Fan
DVM, ACVIM, PhD
Associate Professor of the Department of Veterinary Clinical Medicine
University of Illinois at Urbana-Champaign

"Investigating Direct Procaspase-3 Activating Compounds as Novel Anticancer Agents"

4:30 Closing Reception Room 1707 B&C

KEYNOTE PRESENTATION

Dr. Timothy M. Fan
DVM, ACVIM, PhD

Associate Professor of the Department of Veterinary Clinical Medicine
University of Illinois at Urbana-Champaign

"Investigating Direct Procaspase-3 Activating Compounds as Novel Anticancer Agents"

Resistance to apoptosis endows cancer cells with survival advantages during periods of harsh cellular stress. Mechanistically, cancer cells evade programmed cell death by disrupting the orderly progression of upstream pro-apoptotic signaling pathways, and consequently attenuate procaspase-3 cleavage to caspase-3. Caspase-3 is the primary "executioner" caspase that irrevocably commits cells to apoptosis through the cleavage of over 100 cellular substrates. Therapeutic strategies that target and facilitate procaspase-3 activation, thereby coercing cancer cells to undergo apoptosis despite dysregulated death signaling pathways, have potential to improve cancer treatment outcomes. Through collaborative research with medicinal chemists, several direct procaspase-3 activating compounds have been developed and tested for their tolerability and anticancer activities in murine and canine tumor models as single-agents, as well as in combination with conventional cytotoxic therapies. Our findings support direct procaspase-3 activation as a viable therapeutic strategy and provide a foundational basis for future investigations with procaspase-3 activating compounds as novel anticancer agents, and this seminar will highlight the successes, pitfalls, and opportunities of procaspase-3 activators for the management of cancer.

Dr. Fan is an associate professor of the Department of Veterinary Clinical Medicine at the University of Illinois at Urbana-Champaign. After receiving his Doctor of Veterinary Medicine Degree at Virginia-Maryland Regional College of Veterinary Medicine in 1995, Dr. Fan trained in the clinical disciplines of Internal Medicine at Cornell University and Oncology at the University of Illinois. Following the completion of Dr. Fan's clinical training, he pursued and completed a PhD in Tumor Immunology at the University of Illinois, whereby he investigated the anticancer effects of cytokine manipulation strategies for the treatment of locally-invasive and metastatic tumors in mouse models of disease. Upon completion of his PhD in 2007, Dr. Fan now serves as the principal investigator of the Comparative Oncology Research Laboratory housed in the Small Animal Clinic, Department of Veterinary Clinical Medicine.

Dr. Fan's laboratory works closely with other basic scientists for evaluating novel drugs or drug delivery strategies for the treatment of cancer. Uniquely, Dr. Fan's training as a scientist and veterinarian, has allowed him the opportunity to rapidly investigate and translate novel treatment strategies in dogs with spontaneously-arising cancers, and conduct meaningful comparative oncology research which is hoped to eventually aid in treating cancer in not only companion animals, but also human beings.

SPEAKER ABSTRACTS: MORNING SESSION

Modeling distinct osteosarcoma subtypes in vivo using lineage restricted transgenic shRNA

Tony J Mutsaers*[1], Alvin JM Ng[2], T John Martin[2], Carl R Walkley[2]; [1]Clinical Studies, University of Guelph, [2]St Vincent's Institute, Melbourne, Australia

The recent establishment of genetically defined tractable murine models that are based on the genetics of human disease offer a new means to understand the molecular genetics of osteosarcoma (OS) and further preclinical testing. Faithful models of OS have recently been developed using Cre:lox technology that mirror the fibroblastic subtype. We have now developed a highly penetrant, metastatic model of the osteoblastic subtype through application of lineage restricted transgenic shRNA. This subtype is not represented in current Cre:lox based OS models. This model is highly penetrant and presents with a 75% rate of metastasis with a preference for lung. This approach has also allowed for a direct comparison of the *in vivo* outcome of using the same genetic basis, but different technology, to model OS. This has demonstrated that, at least in this example, the effects of Cre:lox and shRNA mediated targeting of gene expression are qualitatively different and result in distinctive pathology. As such these approaches are highly complementary rather than mutually exclusive in efforts to generate a sufficiently diverse collection of murine models that mirror the diverse pathology of human OS.

Nkd1 is activated by Wnt to prevent nuclear accumulation of β-catenin

Terry Van Raay*, Jahdiel Larraguibel, Rasmeet Dahliwal, Roman Kondra, Joshua Robertson; Molecular and Cellular Biology, University of Guelph

The majority (>80%) of colorectal cancers, and numerous other cancers, are caused by aberrant stabilization of cytoplasmic β-catenin, which is at the core of the Wnt signaling pathway. Stabilized β-catenin translocates into the nucleus to activate the transcription of oncogenes, however, the regulation of cyto-nuclear β-catenin shuttling is poorly understood. Recently, we identified that one obligate and conserved target of the canonical Wnt pathway, Naked Cuticle homologue 1 (Nkd1), functions to inhibit nuclear accumulation of β-catenin, via a negative feedback loop. Through this work we developed a model whereby Wnt signaling induces the expression of Nkd1, which associates with Dvl and is recruited to the plasma membrane. Subsequently, Nkd1 dissociates from Dvl to associate with β-catenin, preventing the nuclear accumulation of β-catenin and thus inhibiting further signaling. One complexity of this model is that Nkd1 function requires its association with the membrane, yet its main function is at the cytoplasm-nucleus interface, preventing nuclear accumulation of β-catenin. We are currently testing the hypothesis that Nkd1 needs to be activated by a Wnt ligand-receptor interaction before it can function as an antagonist. In support of this, we have found that Nkd1 by itself cannot antagonize Wnt signaling induced by other activators, such as a constitutively activated co-receptor. Also, the interaction between Nkd1 and β-catenin is strongest only in the presence of a Wnt ligand. We are also investigating the function of several conserved domains in Nkd1, specifically an EF-hand, which we speculate binds Mg^{++}, and may control Nkd1s function and/or

localization. Finally, Nkd1 is upregulated in most Wnt-induced cancers and our preliminary work in a cancer cell line suggests that if Nkd1 can be activated in these cells, these cells may undergo differentiation.

Evaluating Sites of Post-Translational Modifications in p53 using Convergent Association Testing.

David . K. Y. Chiu* and Ramya Manjunath; School of Computer Science, University of Guelph

In our previous studies involving multiple sequence alignment, we found interdependent (association) sites can be indicative of structural and functional characteristics of the molecule. It can be used in algorithms to construct the tertiary structure of small rRNA and tRNA. Recently, we found information from site associations can be used to construct: 1) the core sites in the 3-dimensional structure of the SH3 protein [Liu & Chiu, 2012], 2) the hierarchical structure of the ubiquitin domains [Durston et al., 2012], and 3) related hereditary patterns of some cancers using p53 [Chiu & Wang, 2004]. In this study, we continue to investigate the roles of sites with association pattern in discriminating between p53 and its homologs. Using the aligned sequences of p53, a tumor suppressor, we develop a new method of association testing that involves the use of multiple level contingency table analysis. The idea is to incorporate analysis using a concept known as granular computing, representing information with different levels of granularity or resolutions. When associations of multiple sites are converged, these sites reflect points of inter-relatedness between sites in the molecules. We found that these identified sites are significantly predictive of post-translational modifications in the molecule, among other things. Furthermore, when these sites are aligned with p63/p73, the homologs of p53, they are statistically discriminating between the human sequences of the p53 family. Thus the study confirms the importance of these identified sites, in addition to other conserved sites of the molecule.

Time for change? An economic evaluation of cervical cancer prevention in Canada.

C.T. Bauch*[1], S.P. Tully[1], A.M. Anonychuk[2,3], D.M. Sanchez[4], A.P. Galvani[4]
[1]University of Guelph, [2]University of Toronto, [3]GlaxoSmithKline Biologicals, [4]Yale University

New technologies, such as human papillomavirus (HPV) vaccines and HPV DNAtesting, are changing the landscape of cervical cancer prevention. Many jurisdictions have implemented universal HPV immunization programs in preadolescent females.However, the cost-effectiveness of modified cervical screening guidelines and/or catch-up immunization in older females in Canada has not been evaluated. Weconducted a cost-utility analysis of screening and immunization with the bivalentvaccine for the Canadian setting from the Ministry of Health perspective. We used adynamic model to capture herd immunity and included crossprotection againststrains not included in the vaccine. We found that adding catch-up immunization tothe current program would be cost-effective, and that combining catch-upimmunization with delaying the age at which screening is first initiated could result incost savings and net health gains.

Paradoxically, immunotherapy can be more efficacious when tumours are inside the brain.

Byram W. Bridle; Pathobiology, University of Guelph

Brain cancer confers a dismal prognosis, necessitating the development of novel treatments. Immunotherapy harnesses the power of the immune system to destroy tumours. Killing with exquisite specificity is a hallmark of immune responses, making this attractive in the brain, where normal tissue cannot be sacrificed. However, central nervous system immunity is stringently regulated by a wide variety of mechanisms that dampen immunosurveillance and heighten immunosuppression. As a consequence, effective brain cancer immunotherapy was not considered feasible until recently.

Using C57BL/6 mice and syngeneic B16-F10 melanoma cells, the impact of cancer vaccines was assessed in the context of tumours transplanted into various anatomical locations. Mice were immunized with recombinant, replication-defective human serotype 5 adenovirus expressing a melanoma-associated antigen with or without boosting with vesicular stomatitis virus encoding the same transgene. Robust tumour-specific $CD8^+$ T cell responses were generated. Efficacy was evaluated in prophylactic versus therapeutic models of cancer in the brain, lungs, skin or subcutaneous compartment. The minimum challenge dose required for 100% tumour engraftment in unvaccinated mice was only 500 cells in the brain versus $1x10^4$ in the skin. Also, prophylactic vaccination was much less effective against intracranial tumour challenge when compared to other anatomical locations (*e.g.* 27% protection against $1x10^3$ B16-F10 cells in the brain versus 100% protection against $1x10^6$ subcutaneous cells). These results support the conventional wisdom that immune responses in the brain are difficult to generate. In stark contrast, vaccination dramatically extended survival in the therapeutic setting only if the tumour was inside the brain. Tumours sequestered in the brain, which are otherwise notoriously immunosuppressive, may be impaired in their ability to down-regulate immune responses induced in secondary lymphoid tissues. If this is true, cancer vaccines might be most effective when tumours are in the brain.

SPEAKER ABSTRACTS: AFTERNOON SESSION

***Fusobacterium nucleatum*: an emerging gut pathogen associated with colorectal cancer.**
Emma Allen-Vercoe; Molecular and Cellular Biology, University of Guelph

Work in my lab focuses on the gut microbiota, and we are particularly interested in bacterial species that may be underappreciated pathogens in the gut niche. In this respect, one area of my research program has focused on a fastidious anaerobic bacterium usually found in the mouth: *Fusobacterium nucleatum*, Fn. This aggregative, pro-inflammatory species has been associated with oral disease, and my group was the first to demonstrate an association between virulent Fn resident in the gut and a type of inflammatory bowel disease: Crohn's Disease. More recently, collaborators at the BC Cancer Agency used high throughput sequencing techniques to determine the microbiome of colorectal cancer (CRC) tumour tissues relative to matched normal tissue, and found a marked over-representation of Fn sequences in the tumour samples, with a positive association with lymph node metastasis. We were able to recover live Fn from a frozen tumour specimen and have characterized some of the properties of this strain. I will discuss this work and our ongoing efforts to uncover the nature of the connection between Fn and CRC.

The Effects of Flaxseed and its Seed Components on Ulcerative Colitis in Female Mice
Leila Zarepoor[1,2], Claire Zhang[1,2], Jenifer Lu[1,2], Krista Power[1,2]*; [1]Human Health and Nutritional Science, University of Guelph; [2]Agriculture and Agri-food Canada, Guelph

Inflammation is involved in the progression of many chronic diseases, including colitis-associated colon cancer (CAC). Flaxseed (FS) is composed of an n-3 fatty acid (n-3-FAs)-rich kernel and a lignan- and fibre-rich hull. Metabolites of lignans and fibre induce anti-inflammatory effects; however, the effects of n-3-FAs are contradictory. The objective of this study was to determine if dietary FS, hull, and kernel can modulate colitis symptoms. Five week-old female C57Bl/6 mice were divided into 4 groups and fed their respective diets for 4 weeks: basal diet (BD); BD+10% FS; 6% kernel; or 4% hull. On day 21, half of the mice were administered 2% dextran sodium sulphate (DSS) in drinking water for 7 days. During the DSS cycle, disease activity index (stool consistency, stool blood, and body weight (BW) loss) was assessed daily. Colon, cecum and spleen were analyzed for biomarkers of inflammation. The proportion and severity of colonic ulcers were measured on H&E-stained sections by morphometrics. FS and hull delayed DSS-induced BW loss and stool blood score increase, while kernel aggravated them. Alternatively, All FS diets increased stool consistency score. At sacrifice, none of the FS diets affected DSS-induced changes in colon and cecum weights, but they all attenuated DSS-induced colon shortening and spleen enlargement. None of FS diets affected the ulcer proportion and severity in the intermediate colon. Furthermore, in non-DSS mice, FS and hull increased cecum content suggesting increased fermentation and short chain fatty acid (SCFAs) production. These results indicate that FS fractions affect colitis differently. The anti-inflammatory effects of FS and hull may be due to the enterolignans and SCFAs production. The adverse effects of kernel on some colitis biomarkers may be related to pro-

inflammatory effects of n-3-FAs. Thus far, this study indicates that interactions between FS bioactives may impact their effects on colitis and potentially CAC.

The Effect of Nicotinamide Supplementation on Caspase-Independent Cell Death in HeLa Cervical Carcinoma Cells.

Dasha Alimenkova, Lindsay Delisle, James B Kirkland*; Human Health and Nutritional Sciences, University of Guelph

Nuclear Poly (ADP) Polymerase (PARP) 1 is a mediator of a caspase-independent cell death pathway, also known as parthanatos. PARP-1 is catalytically activated by DNA strand breaks, leading to rapid production of Poly (ADP) Ribose (PAR) polymers from Nicotinamide Adenine Dinucleotide (NAD+). PAR polymers signal mitochondrial Apoptosis Inducing Factor (AIF) to translocate to the nucleus, where it causes chromatin condensation and large scale DNA fragmentation. This process depends on the availability of NAD+, which is rapidly depleted by PARP-1 during DNA damage. Thus, intracellular levels of NAD+ play an important role in supporting this caspase-independent cell death pathway. In the present study, we hypothesized that increasing intracellular NAD+ levels using nicotinamide supplementation would sensitize HeLa cells to N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)-induced cell death by maintaining PAR production and promoting AIF translocation to the nucleus. Our results indicate that increasing intracellular NAD+ levels in HeLa cells improve MNNG-induced cell death in a dose-dependent manner and this effect strongly correlates with damage-induced PAR production. Overall, these results provide insight into the importance of intracellular NAD+ levels in the caspase -independent cell death and the potential benefit of niacin supplementation in chemotherapy.

Omega-3 fatty acids reduce mammary gland tumour development in MMTV-neuYD5 mice.

K. Perez, M. MacLennan, S. Clarke, W. Muller, J. Kang and D. Ma*; Human Health and Nutritional Sciences , University of Guelph

Introduction. Lifestyle and diet plays a significant role in the prevention of cancer. The role of dietary fat in cancer remains poorly understood. Evidence suggests a potential beneficial effect of n-3 polyunsaturated fatty acids (PUFA); however, a direct cause and effect relationship remains to be established. Therefore, in the present study we have employed complementary genetic and dietary approaches to investigate the role of n-3 PUFA in the prevention of breast cancer. In the genetic arm of the study, the transgenic fat-1 mouse, which is capable of de novo synthesis of n-3 PUFA was crossed with the MMTV-neu-YD5-ndl (MMTV-neu) mouse, a model for breast cancer. Experimental design. Double hybrid progeny, which endogenously synthesize n-3 PUFA and spontaneously develop mammary tumours, were compared to control MMTV-neu mice not exposed to n-3 PUFA. In parallel, MMTV-neu offspring were also fed a fish oil diet (3% w/w) containing n-3 PUFA. Mammary glands were palpated for tumours over a 20 week time-course, tracking volume and multiplicity. Tumour tissue and adjacent mammary

gland tissue were analyzed for fatty acid composition by gas chromatography. Results. Mice exposed to n-3 PUFA from both diet and de novo synthesis exhibited reduced tumour volume and multiplicity, with dietary n-3 PUFA having greater beneficial outcomes compared to endogenously made n-3 PUFA. However, n-3 PUFA had no effect on the age of first tumour onset or the rate of tumour growth per day. Summary and conclusion. Lifelong exposure to n-3 PUFA , whether by dietary or genetic means, was shown to be beneficial in reducing mammary tumour development and may play an important role in breast cancer prevention.

Palliative Treatment of Osteosarcoma in Dogs: What should we do?

Sarah Boston; Clinical Studies, University of Guelph

Bone cancer pain is an extremely painful condition that is difficult to treat. Based on murine models of bone cancer, bone cancer pain appears to be a multifactorial condition that is a separate entity from both inflammatory and neuropathic pain. The approach to managing bone cancer pain in the palliative setting for osteosarcoma in dogs must also be multifactorial. It should involve analgesia, treatments that kill tumor cells (radiation and chemotherapy), treatments that decrease osteoclast activity and possibly mechanical support. Radiation therapy is a cornerstone of bone cancer pain management. There are several protocols in the veterinary literature for the palliative management of bone cancer pain, without a definitive study to show which protocol has the greatest efficacy. A study using force plate analysis is currently underway to evaluate the efficacy of radiation therapy in dogs with OSA. Bisphosphonates are used in palliative protocols to manage osteosarcoma in dogs. A recent retrospective study by our group showed that the addition of pamidronate into a palliative radiation protocol significantly decreased survival time in canine osteosarcoma patients. A small pilot in vitro study echoed these results in osteosarcoma cell lines, and showed that cell counts were higher when osteosarcoma cells were treated with radiation and pamidronate, compared to radiation alone. These findings are counterintuitive to current theory, but highlight that more study is needed to understand when bisphosphates should be used for palliation of bone pain. The addition of chemotherapy into a palliative radiation protocol has been shown to increase survival in dogs by our group and other studies. We are currently investigating a novel therapy, chemoembolization to manage OSA in dogs. Mechanical stabilization of impending or actual pathological fractures is controversial, but a retrospective study has shown that in select cases, long-term survival and palliation can be achieved.

Basophilic Leukemia in a Standardbred Gelding.

Cathy Furness[1], Emilie Setlakwe[1], Darren Wood[2]* and Luis Arroyo[1]*; [1]Department of Clinical Studies; [2] Pathobiology, University of Guelph

A three-year-old Standardbred gelding was evaluated for a history of pyrexia, uncontrolled bleeding from the oral cavity and a large, soft swelling at the junction of the caudal aspect of the rami and proximal neck. Upon examination, there was evidence of active bleeding from the oral cavity, previous venipuncture sites, the abdomen was grossly distended and there was distension

noted in multiple joints. The gelding was moderately tachycardic and had pale mucous membranes. An abdominal ultrasound revealed a large volume of free fluid. Abdominocentesis was performed which confirmed frank hemorrhage into the abdominal cavity. An aspirate was collected from the soft swelling which also confirmed hemorrhage. The centesis site bled for approximately 48 hours following the procedure. A complete blood count performed on admission revealed a leucopenia characterized by a moderate neutropenia and a low hemoglobin concentration. The results of coagulation profiles were prolonged, including prothrombin time and the activated partial thromboplastin time. Significant elevations in D-dimer concentration and fibrin degradation products titre, as well as a markedly low fibrinogen concentration were strongly suggestive of DIC. Supportive care included antibiotic therapy, vitamin K supplementation and plasma and whole blood transfusions. A bone marrow aspirate and biopsy was performed which revealed a predominance of immature myeloid cells with basophilic granules. A basophilic leukemia was diagnosed and confirmed on post mortem examination. Basophilic leukemia is a rare form of acute myeloid leukemia which has been reported in humans, cats, dogs and cattle; however, this is the first report of basophilic leukemia in a horse.

Factors associated with the recommendation of a referral to specialty oncology services.

Stoewen DL*[1], Coe JB [1], MacMartin C [2], Stone EA[3], Dewey CE [1]; [1]Population Medicine, [2]College of Social and Applied Human Sciences, University of Guelph

An interview-based qualitative study exploring the expectations of clients identified that some clients had difficulty in accessing the specialty oncology services offered at the Ontario Veterinary College because their family practitioner lacked awareness of what the service could offer and achieve, was less than optimistic about their dog's cancer and/or its treatment, or demonstrated a less than optimal tendency to refer. Ten percent (3/30) had needed to seek a second opinion to get a referral, and concern was expressed for the ability of other dogs and their families to access care. In response, a vignette-based survey study of primary care practitioners was undertaken to identify the treatment recommendations of practitioners with 2 types of life-threatening cancers in dogs: multicentric lymphoma and appendicular osteosarcoma. Following logistic regression analysis, the factors associated with the recommendation of a referral were determined. According to our findings, practitioners are more likely to recommend a referral when their patient is in good health, their client is strongly bonded and financially secure, they lack expertise with cancer treatment but consider it worthwhile, and are completely confident in the referral centre, with some variability in relation to their gender and the type of medicine they practice. In sum, practitioners' propensity to recommend a referral is not simply related to diagnostic uncertainty and treatment complexity, as recognized within the literature, but is contextually multifactorial, taking into account the self-as-practitioner-, client-, and patient-related factors.

POSTER ABSTRACTS

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1)

Investigating the role of Nck adaptor proteins in breast cancer.

Una Adamcic and Nina Jones*; Molecular and Cellular Biology, University of Guelph

Invasive metastatic mammary carcinoma cells form small membrane protrusions, called invadopodia, that allow them to penetrate basement membranes of blood vessels, and aid in tumor cell invasion and metastasis. Invadopodia are enriched with actin filaments and signaling proteins such as the Nck family of intracellular adaptors that regulate the actin cytoskeleton. Remodeling of actin occurs during invasion as a response to extracellular signals that initiate cascades of intracellular signaling pathways eventually leading to cellular migration. Given the established role of Nck proteins in cell migration, the goal of this study is to investigate whether Nck signaling may be required for breast cancer progression. *In vitro* studies are currently being performed to measure cell survival, proliferation and invasion in two breast cancer cell lines (MCF-7 and MDA-MB-231) treated with silencing shRNA to knockdown Nck1/2 protein levels. Future *in vivo* studies will examine the ability of these Nck knockdown cell lines to grow and invade tissues upon xenografting them into immunodeficient mice. Tumor growth and potential lung metastasis will be measured in these animals and compared to controls to establish the effect of Nck adaptor proteins on breast cancer growth and progression. Additional *in vivo* studies will be performed utilizing Cre/LoxP technology to determine the effect of mammary epithelial-specific deletions of Nck1 and Nck2 on development of mammary tumors in breast cancer mouse model. To date, there is no direct *in vivo* evidence linking Nck expression to breast cancer, thus these studies will provide better insight into the role of Nck cytoskeletal adaptor proteins in mammary tumour initiation and progression.

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2)

Involvement of Glutathione S-Transferase A1 in controlling Proliferation, Differentiation and Apoptosis in Human Colonic Adenocarcinoma Caco-2 cells.

Humaira Adnan, Monica Antenos, and Gordon M. Kirby*; Biomedical Sciences, University of Guelph

The colonic epithelium continuously regenerates with transitions through various cellular phases including proliferation, differentiation and cell death via apoptosis. Human colonic adenocarcinoma (Caco-2) cells in culture undergo spontaneous differentiation into mature enterocytes in association with progressive increases in expression of the alpha class glutathione S-transferase (GSTA1). We hypothesize that GSTA1 plays a functional role in controlling proliferation, differentiation and apoptosis in Caco-2 cells. We demonstrate increased GSTA1 levels associated with decreased proliferation and increased differentiation markers alkaline phosphatase, villin, dipeptidyl peptidase-4 and E-cadherin in postconfluent Caco-2 cells. Results of MTS assays, BrdU incorporation and flow cytometry indicate that forced expression of GSTA1 significantly reduces cellular proliferation and siRNA-mediated knock-down of GSTA1 significantly increases cells in S-phase and associated cell proliferation. Sodium butyrate at a concentration of 1 mM reduces Caco-2 cell proliferation, increases differentiation and increases

GSTA1 activity. In contrast, 10 mM NaB causes significant toxicity in preconfluent cells via apoptosis through caspase-3 activation with reduced GSTA1 activity. GSTA1 knock-down by siRNA does not alter NaB-induced differentiation or apoptosis in Caco-2 cells. While 10 mM NaB causes GSTA1-c-Jun N-terminal kinase (JNK) complex dissociation, phosphorylation of JNK is not altered. These findings suggest that GSTA1 levels may play a role in modulating enterocyte proliferation but do not influence differentiation or apoptosis.

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3)

The role of MLH1 and MSH2 in tumor growth and drug resistance in human colorectal cancer cells.

Amanda Barber, Kristen Lacombe, Brenda L. Coomber*; Biomedical Sciences, University of Guelph

Loss of genomic stability is associated with a variety of diseases, most notably cancer. Of the many proteins involved in maintaining genomic integrity, two of the most important are MLH1 and MSH2, which are responsible for repairing single-base mismatches and insertion and deletion mutations. Loss of these two proteins leads to a cancerous phenotype known as microsatellite instability, which is observed in approximately 15% of all colorectal cancers. Previous work established derivatives of the CaCo2 human colorectal cancer cell line with siRNA-mediated knockdown of these proteins. These cells were then transfected into mice, and tumors grew much faster than controls, implicating the importance of these genes in maintaining the genome and preventing the development of neoplasms. Following growth *in vivo*, clonal cell lines were established from the tumors and used to examine the effects that knockdown of MLH1 and MSH2 had on other members of the DNA mismatch repair system proteins. Following loss of MSH2, cells also experienced loss of one of its major binding partners, MSH3. It is expected that MSH6, another MSH2 binding partner, will undergo a similar phenomenon. Clonogenic survival following exposure to the chemotherapeutic drug 5-fluorouracil was also evaluated, and those cells with reduced MLH1 and MSH2 levels appear to be resistant to this drug, implicating the importance of evaluating mismatch repair status prior to deciding on a course of treatment for a given patient.

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4)

The influence of *Fusobacterium nucleatum* in synergistic infections.

Kyla Cochrane, Jaclyn Strauss, Michelle Daigneault, Emma Allen-Vercoe*; Molecular and Cellular Biology, University of Guelph

The intestinal microbiota is a dynamic ecosystem of emerging importance to human health. Bacterial species within this ecosystem have co-evolved with their host to allow for the maintenance of homeostasis and to avoid imbalances in microbial populations which in turn may lead to a diseased state in the host. The search for members of the microbiota that are capable of driving inflammatory responses in the host is a current research focus. In this respect Fusobacterium nucleatum (Fn) is a potential candidate member of the normal intestinal microbiota with pro-inflammatory attributes, and this species has recently been specifically

associated with inflammatory bowel disease and colorectal cancer. Fn is an invasive pathogen that is also known to co-aggregate with a wide variety of different bacterial species. It has been shown that, through such co-aggregation, Fn can directly facilitate internalization of other normally non-invasive bacterial species in vitro. Such a phenomenon offers a potential mechanism whereby luminal bacterial species can come into direct contact with the host epithelial immune system, which may in turn contribute to an inflammatory response. If this is the case, then the involvement of Fn in disease could be a function of the microbes to which it aggregates, rather than a direct result of its own virulence determinants. We have found that different Fn isolates have distinct binding trophisms for particular secondary bacterial binding partners isolated from the human gut. This study aims to examine the implications of such co-aggregation on co-invasion by Fn, and the resulting downstream effects on the stimulation of inflammatory markers and/or enhancement of invasion capabilities of bound bacterial species.

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5)
Investigation of potential JSRV envelope interacting proteins.
Jondavid de Jong, Sarah K. Wootton*; Pathobiology, University of Guelph

Jaagsiekte sheep retrovirus (JSRV) is the causative agent of ovine pulmonary adenocarcinoma (OPA), a naturally occurring transmissible lung cancer of sheep. The closely related Enzootic nasal tumour virus (ENTV) is thought to be the causative agent of nasal tumours in sheeps and goats. Unlike most oncogenic retroviruses, the virally encoded JSRV and ENTV envelope (Env) proteins are potent oncogenes. Expression of Env alone is sufficient to induce transformation in a variety of cell types. In vitro Env expression results in the activation of the PI-3K/Akt and Ras-MEK-MAPK signal transduction pathways. The cytoplasmic tails of both JSRV and ENTV Env encode putative binding sites for the p85 subunit of PI-3K (YXXM) and the tyrosines have been implicated in transformation, however, the tyrosine residues do not appear to be phosphorylated during transformation as would be expected. Additionally, there is no evidence to support binding of the p85 subunit to Env. In order to elucidate other potential Env binding partners, JSRV Env was utilized as bait in a split ubiquitin membrane protein yeast two-hybrid assay. This poster will summarize our preliminary data, including co-IP assays, and co-localization using confocal microscopy, in confirming JSRV Env interaction with selected cancer related "hits" from the yeast two-hybrid analysis.

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6)
Effect of DCA treatment on Bcl-2 family protein expression in colorectal cancer cells.
Leanne Delaney, Brenda Coomber*; Biomedical Sciences, University of Guelph

Recent evidence has supported the use of dichloroacetate (DCA) as a cancer-specific therapy that targets the unique metabolism of cancer cells but not normal somatic cells. However, our laboratory has shown that some human colorectal cancer cell lines may not respond to treatment as predicted, and the mechanism by which DCA alters the behavior of these cells is currently unknown. Of interest in the investigation of DCA's effectiveness on these cancer cell lines is the

Bcl-2 protein family, which is involved in cell death pathways. The Bcl-2 proteins have in common several domains, the combination of which determines whether apoptosis will be promoted or prevented. The expression, regulation, and interactions of the Bcl-2 proteins will ultimately determine whether the outer mitochondrial membrane will be compromised, allowing the release of cytotoxic molecules into the cell. These cytotoxic molecules, such as cytochrome c, will activate a caspase cascade, which will result in DNA fragmentation, membrane blebbing, and apoptosis. Post-translational regulation of the Bcl-2 protein Mcl-1 is of interest as Mcl-1 is anti-apoptotic until cleaved by caspase 3, at which point it exhibits pro-apoptotic characteristics. Using cell culture and western immunoblotting, we examined key changes in members of the Bcl-2 family members in response to DCA treatment in human colorectal cancer cell line HCT116 under hypoxic and normoxic conditions. Preliminary results suggest that the presence of a potentially pro-apoptotic as well as an anti-apoptotic version of Mcl-1 is diminished when cells are treated with DCA. The interactions that could be involved in this change are currently under investigation. Further research may provide insight into the mechanism by which some human cancer cell lines are protected against DCA-induced cell death, and the potential role of intrinsic apoptotic pathway regulation in this cell-specific protection.

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7)

The effect of colonosphere formation on proteins involved in cancer stem cell sustainability pathways.

Nathan Ferias, Brenda Coomber*; Biomedical Sciences, University of Guelph

Recent findings support the theory that a proportion of tumor cells, termed cancer stem cells, share properties with native stem cells and are vital in the development and perpetuation of tumor growth and metastasis. Particularly, colorectal cancer may be especially vulnerable to this hierarchal tumor organization due to the continuous crypt homeostasis, which is responsible for the renewal of the intestinal epithelial lawyer. Consequently, this may put us at serious risk of developing cancer. In an attempt to enrich for cancer stem cells we grow cells under non-adherent conditions and supplement them with stem cell growth factors. Using these specialized cell culture techniques we have assessed the capacity of weakly tumorigenic and highly tumorigenic human colorectal cancer cells lines, Caco2 and HCT116 respectively, as well as a non-tumorigenic rat intestinal epithelial cell line, IEC18, to enrich for cancer stem cells and form colonospheres in vitro. Our results show that HCT116 cells form colonospheres at a relatively higher efficiency and quality (93.9% colonopheres formed) compared to Caco2 (38.2% colonospheres formed), while IEC18 cells failed to form colonospheres at all. We then compare the expression of proteins involved in critical stem cell sustainability pathways between the parental and stem cell enriched cell lines via western blot analysis. Particularly, we looked at proteins involved in Wnt, Notch, TGFβ and DNA methylation associated pathways, which consist of Axin1, GSK3β, β-catenin, Nodal, Notch1, DNMT-1 and EpCAM. Thus far, our research shows that the ability to enrich for cancer stem cells is unique to our different cell line models and the intracellular signaling pathways that are involved in maintaining stemness are up regulated in cancer stem cells. Through this research we hope to elucidate the main pathways involved in maintaining colorectal cancer stem cells and the mechanisms that are contributing to the activation of these pathways.

8)

Hyperglycemia accelerates the progression of epithelial ovarian cancer in mice, which may be mediated by active sodium-glucose co-transport.

Lisa Kellenberger[1], Alison Holloway[2], and Jim Petrik*[1];[1]Biomedical Sciences, University of Guelph, [2]Obstetrics and Gynaecology, McMaster University

Hyperglycemia is now a common comorbidity with epithelial ovarian cancer (EOC) and is associated with poor cancer prognosis. Since circulating glucose is the primary source of metabolic fuel for transformed cells, we hypothesize that with increased availability, glucose will accelerate the growth of ovarian tumors. Using an orthotopic, syngeneic model of EOC we induced tumor growth in hyperglycemic mice in the absence or presence of hyperinsulinemia: we administered streptozotocin to destroy pancreatic β-cells in wildtype mice resulting in high glucose with low circulating insulin; and we used hyperglycemic AKT2 -/- mice with impaired insulin sensitivity and elevated insulin levels. In both the insulin (-) and insulin (+) hyperglycemic models, tumor growth was significantly accelerated by 90 days compared to normoglycemic mice and there was a strong correlation between baseline blood glucose and tumor size. *In vitro* we have shown that murine EOC cells (ID8) express both passive glucose transporters (GLUT) and active sodium-glucose co-transporters (SGLT). Blocking the uptake capacity of the GLUT family using the competitive inhibitor phloretin significantly reduced cell viability, glucose consumption and invasive capacity. The SGLT inhibitor phlorizin led to a decrease in cell viability and glucose consumption, particularly during acute exposure (48h) to elevated glucose. Furthermore, cells chronically cultured in hyperglycemic environments were more invasive and more sensitive to SGLT inhibition than cells acclimatized to normoglycemic conditions. Together, these data show that glucose alone is sufficient to accelerate ovarian tumor growth and the SGLT transporters may be novel mediators of tumor fuel acquisition in hyperglycemic environments.

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9)

Chronic low-grade, systemic inflammation accelerates the progression of epithelial ovarian cancer (EOC) in vivo.

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

An inflammatory environment can enhance tumorigenesis, however specific mechanisms are not well understood. Epidemiological data have described a link between chronic inflammatory diseases, such as diabetes or obesity, and epithelial ovarian cancer (EOC) suggesting that systemic inflammation may potentiate the risk of EOC. The purpose of this study was to identify the impact of prolonged exposure to chronic low-grade inflammation on EOC tumor progression in vivo. We hypothesized that exposure to systemic chronic inflammation would enhance the growth of EOC tumors by increasing cell survival, angiogenesis and metastatic capability. Prior to tumor initiation, chronic low-grade inflammation was induced in one group of C57Bl-6 mice via daily intraperitoneal injections of bacterial endotoxin lipopolysaccharide (LPS) while the control group received daily PBS injections. Ovarian tumors were induced with an orthotopic injection of tumorigenic murine ovarian surface epithelial (ID8) cells. At both 60 and 80 days

post tumor-initiation (PTI) ovarian tumors from LPS treated mice were significantly larger than vehicle treated controls. In a second cohort, we performed a survival experiment and found that due to accelerated tumorigenesis, LPS-induced systemic inflammation resulted in a decreased time to death compared to controls. Evaluation of the relationships between chronic systemic inflammation and EOC may lead to the development of anti-inflammatory treatment approaches and could provide insight into the role of inflammation in the progression of other human cancers. Additionally, as the rate of metabolic disorders increases in the Western world the results from this work may facilitate the advancement of complimentary therapeutic interventions for other related cancers.

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10)

Additional local therapy with primary re-excision or radiation therapy improves survival and local control after incomplete surgical excision of mast cell tumours in dogs.

K. Kry; S. Boston*; Clinical Studies, Univeristy of Guelph

Mast cell tumors are the most common cutaneous neoplasia in dogs. The recommended primary treatment is surgical excision with appropriate margins, but there is debate as to the approach for cases with incomplete resection. The purpose of this study is to compare survival and local recurrence outcomes in dogs with incompletely resected mast cell tumours treated with primary re-excision or radiation therapy of the primary site versus no additional local therapy. The hypothesis of this study is that additional local therapy will provide improved local disease control and prolonged survival outcomes compared to when no additional local therapy is pursued following incomplete mast cell tumor resection. This was a retrospective study of 70 cases of canine mast cell tumours in 64 dogs evaluated at the Ontario Veterinary College Teaching Hospital from 2001-2010 that had previous incomplete surgical resection. Outcome was evaluated with additional local therapy (either primary re-excision or radiation therapy) or no additional local therapy (comparison group). Follow-up was performed through evaluation of medical records, and telephone contact with referring veterinarians and owners. Median survival time for the primary re-excision group was 2930 days, for the radiation therapy group was 2194 days and for the comparison group was 710 days. Local recurrence was noted in 3 cases (13%) of the re-excision group, 2 cases (8%) of the radiation therapy group and 10 cases (38%) of the comparison group. Notable treatment complications were seen in 5 (22%) of the re-excision cases and 19 (90%) of the radiation therapy cases. A history of previously completely resected mast cell tumours did not have an effect on overall survival. There is significant improvement in survival and local control when additional local therapy is performed following incomplete resection of mast cell tumours.

11)

Investigating the Role of Wnt8 on Nkd1's Activation and Function.

Jahdiel Larraguibel, Terry Van Raay*; Molecular & Cellular Biology, University of Guelph

Wnt signaling is a highly conserved pathway found in both invertebrates and vertebrates. This conservation is due to its 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. In the majority of colorectal cancers, up to 85%, there is elevated Wnt activity due to mutations in the Wnt signaling pathway that result in its constitutive activation. The most common mutation is found in the scaffolding protein Adenomatous Polyposis Coli (APC), which functions in maintaining low levels of Wnt activity. Although Wnt activity induces its own negative feedback regulators Naked1 (Nkd1) and Axin2, preliminary data suggests Nkd1 is not activated without a Wnt ligand present. In individuals with APC loss of function, there is elevated Wnt activity and subsequent expression of Nkd1 but not activation due to the absence of a Wnt ligand. Therefore, fully elucidating the signaling events of Nkd1 activation will have important consequences in understanding how misregulation of this pathway contributes to cancer. To study Nkd1 activation, varying Wnt agonists will be injected into early zebrafish embryos with or without *nkd1* or *wnt8*, a Wnt ligand. By using various Wnt agonists that stimulate the pathway at multiple levels, it can be reasoned whether or not Nkd1 activation is directly related to the presence of a Wnt ligand or just elevated Wnt signaling. The level of Wnt activity will be assessed using Whole-Mount *in situ* hybridizations probing for target genes of Wnt expression. Ectopic expression brought on by the Wnt agonists should be rescued by the co-injection of *nkd1* and *wnt8* together. This information may prove to be quite valuable in evaluating how Naked1 is activated and how activating this endogenous negative feed-back regulator can aid in preventing and treating colorectal cancers.

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12)

The Effects of Purified Rutin and Rutin-Rich Asparagus on Colonic Inflammation in C57BL/6 Mice.

Jenifer Lu[1,3] Claire Zhang[1,3], Leila Zarepoor[1,3], David Wolyn[2], Rong Tsao[2,3], Krista Power[1,3]*;[1]Human Health and Nutritional Sciences and [2]Plant Agriculture, University of Guelph; [3]Guelph Food Research Center, Agriculture and Agri-Food Canada

Inflammatory bowel disease (IBD) is a major risk factor for colitis-associated colon cancer. Studies show that purified rutin (RUT), a flavonoid glycoside in many fruits and vegetables, reduces IBD symptoms. Currently, it is not known whether the anti-inflammatory effects of RUT can be achieved through consuming RUT-rich whole foods. This study will compare the effects of RUT and cooked RUT-rich asparagus (ASP) on colitis in mice to determine the role of ASP as an alternative therapy for IBD. The hypothesis is that the beneficial effects of RUT will be obtained by consuming RUT-rich ASP. C57BL/6 male mice were fed a basal diet (BD) with or without 6% ASP flour or 0.05% RUT (equivalent RUT in 6% ASP) for 4 weeks. In the last week, ASP and RUT groups, and half of the BD group were exposed to 2% dextran sulfate sodium (DSS) in drinking water. Mice were monitored daily for food and water intake, and disease activity index (DAI) (body weight (BW) loss, stool consistency, and stool blood). Colon, cecum, and spleen were assessed for biomarkers of inflammation, and the colon was analyzed for

myeloperoxidase (MPO) levels, indicative of colonic neutrophil infiltration. RUT significantly delayed BW loss and had no effect on diarrhea onset, while ASP aggravated both. On the other hand, ASP attenuated DSS-induced stool blood score. Both groups attenuated DSS-induced colon shortening and had no effect on cecum and spleen weights, yet ASP alone increased colon weight. Colon MPO levels were neither attenuated nor aggravated with either diet. Overall, consumption of RUT and RUT-rich ASP were effective in reducing some biomarkers of inflammation. Potential reasons why ASP aggravated some symptoms of colitis (DAI and colon weight) is unknown; however, other ASP components could be intereferring with the beneficial effects of RUT.

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13)

The role of the CDP-ethanolamine pathway in autophagosome formation.

L. Pereira, V.M. Gohil, M. Bakovic*; Human Health and Nutritional Sciences, University of Guelph

Autophagy, the process that degrades cytosolic constituents into products that can be recycled for use in energy generation, occurs at basal levels in all cells and may be upregulated during periods of stress. Autophagosome formation is a necessary development during macroautophagy. Yet the origin and source of the autophagosomal membrane remains to be fully described. The endoplasmic reticulum is responsible for the bulk synthesis of the phospholipids phosphatidylethanolamine (PE) and phosphatidylcholine (PC) via two independent branches of the Kennedy pathway: the CDP-ethanolamine and the CDP-choline pathway, respectively. To examine the role of the Kennedy pathway in autophagosome formation, a cell line deficient in Pcyt2, a gene that encodes the rate-limiting enzyme CTP-ethanolamine cytidyltransferase (ET) in the CDP-ethanolamine pathway, was constructed in immortalized human fibroblasts using lentiviral shRNA constructs targeting Pcyt2. Pcyt2 silencing was confirmed by quantitative real-time PCR and Western blot analysis. Interestingly, Western blot analysis under normal and starvation conditions showed reduced expression of both LC3-I (microtubule-associated protein 1 light chain 3) and LC3-II in Pcyt2 knockdown cells during starvation (induced by Earle's Balanced Salt Solution). Through [14C]-ethanolamine radiolabeling experiments, Pcyt2 knockdown cells showed reduced de novo PE synthesis (0-2 hours), but increased PE synthesis after 2 hours under starvation conditions as compared to non-starvation conditions. Whereas under starvation, control cells showed reduced PE synthesis after 2 hours. Pulse-chase experiments using [14C]-ethanolamine revealed utilization of PE (1-2 hours) and re-synthesis after 2 hours during starvation-induction of autophagy in Pcyt2 knockdown cells. In comparison, control cells showed faster degradation of PE (0.5-1 hours) and then slower rates of re-synthesis of PE (1-3 hours) under starvation conditions. Currently we are investigating the role of the Kennedy pathway in autophagosome formation using various tissues collected from fasted control and Pcyt2 knockdown mice.

14)

Characterization of a Novel Retroviral Envelope-Based Mouse Model for Human Lung Cancer.

Nicolle Petrik, Sarah Wootton*; Pathobiology, University of Guelph

Lung Cancer remains the leading cause of cancer related deaths with a 15% survival rate over a 5 year period. Pulmonary adenocarcinoma is the most prevalent non-small cell lung cancer (NSCLC) in both men and women. The main risk factors for lung cancer include cigarette smoke, asbestos, environmental pollution and radiation. However, about 15% of all lung cancer cases are not attributed to any of these risk factors. It has been estimated that 15–25% of human cancer may have a viral etiology and two viruses in particular, the human papilloma virus (HPV) and Jaagsiekte sheep retrovirus (JSRV), have been speculated to have a role in the pathogenesis of lung cancer. The objective of this research is to better understand JSRV involvement in human lung cancer and understand the molecular mechanisms of JSRV envelope (Env) induced lung tumours in mice. We have identified JSRV Env expression in human lung tumor tissue arrays, specifically in pulmonary adenocarcinomas and squamous cell carcinomas. JSRV Env expression was observed in all stages of the disease indicating that it could be involved in both tumor initiation and development. To further our understanding of the molecular mechanisms involved in JSRV Env-induced oncogenesis we have characterized the cell signaling pathways activated within JSRV-Env induced lung tumors. This research represents a comprehensive investigation into the function of the JSRV Env protein in lung tumorigenesis.

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15)

Investigation of the role of hindsight in modulating Notch signaling in *Drosophila*.

Bruce H. Reed*, Brittany Baechler, Yang Du; Biology, University of Waterloo

The Drosophila homolog of the human Ras Responsive Element Binding Protein-1 (RREB1), a zinc finger transcription factor implicated in some forms of prostate cancer, is encoded by the gene hindsight (hnt). In Drosophila hnt has been identified as a target of the Notch signaling pathway and is required for the regulation of epithelial organization as well as the transition from the mitotic cell cycle to the endo-cycle. To better understand the role of hnt in the Notch signaling pathway we have used the GAL4/UAS system of inducible gene expression to perform a microarray analysis of stage 12-14 embryos over-expressing hnt. Candidate genes that are up-regulated in the background of global hnt-overexpression include several known Notch signaling antagonists. To validate the role of hnt in modulating the cellular response to Notch signaling, we are also examining the differentiation of peripheral cells within the clusters of adult midgut precursor cells in the larval midgut. Peripheral cells form a transient stem cell niche and we will present evidence that suggests a role for hnt in the Notch dependent differentiation of peripheral cells.

16)

Investigating the dose-dependent signaling of TGFβ.

Amy Richard, Alicia Viloria-Petit*; Biomedical Sciences, University of Guelph

Transforming growth factor-beta (TGFβ) is an important signaling molecule, which regulates several cellular processes including angiogenesis. However, its effects on angiogenesis are complex, with TGFβ promoting angiogenesis only at low concentrations. Evidence suggests that downstream signaling pathways of TGFβ may be activated in a dose-dependent fashion. In fact, the Viloria-Petit lab has shown that the Par6 polarity pathway gets preferentially activated at low concentrations. Considering the different cellular effects of downstream signaling pathways, we propose that TGFβ may modulate its effects on angiogenesis via differential activation of the canonical Smad and non-canonical Akt, FAK and Par6 polarity signaling pathways. Based on this premise, Bovine aortic endothelial cells were treated with a range of TGFβ1 concentrations, from 0.01 to 5 ng/mL. The activation patterns of canonical and non-canonical signaling pathways were studied via Western blotting; with the use of phospho-specific antibodies against Smad2, Akt and FAK. Preliminary results reveal that high concentrations (5 ng/mL) cause preferential activation of Smad2, while the FAK and Akt signaling pathways do not appear to become activated in response to TGFβ1 at the concentrations studied. These results suggest the effect of TGFβ on angiogenesis may not involve Akt or FAK signaling, but may involve dose-dependent signaling of the Smad signaling pathway.

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17)

Wnt regulators dickkopf1 and naked1 act synergistically to antagonize canonical wnt signaling.

Joshua Robertson, Terry Van Raay**.** Molecular and Cellular Biology, University of Guelph

Adult stem cells are keystone to organ function and their inadequate maintenance and over-proliferation is often linked to the onset of cancer. At the center of stem cell homeostasis is the Wnt signaling pathway and upon activation induces the cytoplasmic stabilization and subsequent nuclear translocation of the transcriptional co-activator, β-catenin, which maintains expression of the stemness genes. However, outside of the stem cell niche, aberrant Wnt signaling and β-catenin translocation is carcinogenic and at the root of >80% colorectal cancers. Wnt signaling also induces the expression of negative feedback regulators such as the cytoplasmic protein Nkd1, which acts to inhibit the nuclear localization of β-catenin, and Dkk1, which acts extracellularly to inhibit Wnt ligand binding to its co-receptor LRP6. Interestingly, there is high expression of both Dkk1 and Nkd1 in various cancers. Though they antagonize Wnt signaling at different functional levels, preliminary experiments have revealed a potential interaction between Dkk1 and Nkd1. Here, we investigated the hypothesis that Nkd1 and Dkk1 act synergistically to inhibit canonical wnt signaling. A low dose of Dkk1 overexpression in zebrafish results in a larger head, while Nkd1 overexpression has no phenotype. However, co-expression results in an enlarged head with absolutely no tail. To further investigate this interaction we looked at the expression of Wnt target genes early in development and found that the combination of Dkk1 and Nkd1 dramatically inhibits Wnt signaling involved in patterning the embryo, but it is not clear if there is synergy. From this, we can begin to develop a model whereby the extracellular

activity of DKK1 influences the intracellular activity of Nkd1 to regulate Wnt signaling and start to address why these two antagonists cannot control abberant Wnt signaling in disease.

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18)

Ectopic IL-11 Expression Enhances Lethality in a Murine Model of Ovarian Cancer.

Negin Sardashti, Timothy Gingerich, Nicole Campbell, James Greenaway, James J. Petrik, Jonathan LaMarre*; Department of Biomedical Sciences, Univrersity of Guelph

Ovarian cancer is a serious gynecological malignancy with over 2600 diagnoses, and 1750 deaths annually in Canada. Epidemiological evidence points strongly to associations between inflammation occurring during ovulation and disease development. One proposed mechanism by which inflammation induces tumor formation is through the activities of cytokines that are present in inflammatory environments. IL-11 is one such cytokine that initiates signaling by binding to its receptor, IL-11Rα and to the signal-transducing receptor gp130. IL-11 receptors are present on over 95% of all human ovarian cancers. We have examined the effects of IL-11 on the behaviour of ovarian cancer cells in an orthotopic model using the ID8 murine ovarian tumor cell line. Three different ID8 clones stably expressing high levels of IL-11 relative to untransfected controls were isolated and used to generate tumors by ovarian bursal injection in mice. Tumors from all clones ectopically expressing IL-11 showed significantly shorter survival times and greater rates of ascites development than those with control ID8 cells. IL-11 expression levels were inversely correlated to survival times, and mice bearing tumors from ID8 Clone 25, which expressed the highest levels of IL-11 mRNA and protein, survived half as long as controls. IL-11 receptor mRNA (IL-11R a and gp130) was present in both untransfected and IL-11 expressing ID-8 cells. Studies are currently underway to confirm the surface expression of these receptors in the tumors. These studies suggest that the expression of IL-11 increases aggressive cellular behaviour in an ovarian tumor model. (Supported by CIHR).

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19)

Do epigenetic enzyme inhibitors enhance the effects of anti-angiogenic therapy on human endothelial cells?

Karolina Skowronski and Brenda Coomber*; Biomedical Sciences, University of Guelph

DNA methylation and post-translational histone modifications are important regulators of gene expression and are catalysed by DNA methyltransferase (DNMT) and histone deacetylase (HDAC) enzymes, respectively. While these inhibitors have been extensively studied in cancer cells, their effects on the tumor's endothelium have only briefly been explored. We sought to learn how these epigenetic inhibitors will impact endothelial cell behaviour, and if combined with a targeted anti-angiogenic therapy, sunitinib, whether the inhibition of angiogenesis could be enhanced. Unexpectedly, while sunitinib inhibited VEGFR2 activation at Y951 in a time and dose-dependent manner in HUVECs, the key residue Y1175 was not dephosphorylated by any dose. When downstream signalling pathways were analysed, a decrease in AKT phosphorylation was seen after 30 minutes of sunitinib treatment, however, AKT was rephosphorylated by 24

hours post treatment. Apoptosis was induced in a time- and dose-dependent manner, except following 24 hours of treatment, where caspase-3 cleavage was decreased compared to shorter treatment times. HUVEC proliferation was not significantly inhibited by any dose of sunitinib, possibly due to the lack of dephosphorylation of Y1175. When the DNMT inhibitor 5-aza-2'-deoxycytidine (5-aza-dC) was combined with sunitinib, inhibition of HUVEC proliferation was enhanced compared to treatment with sunitinib alone. HUVEC migration was also inhibited by the combination of these two inhibitors, but not in an additive manner. Although the HDAC inhibitor Trichostatin A did significantly decrease HUVEC proliferation and migration, there was no additive effect when combined with sunitinib. This study revealed that combining a DNMT inhibitor with sunitinib is more effective on decreasing endothelial cell proliferation than with a HDAC inhibitor. The impact of treatment with sunitinib and 5-aza-dC on tumor angiogenesis and tumor growth remains unknown, and in vivo trials will need to be performed to determine if combining these two agents will enhance anti-angiogenic activity.

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20)

What we can learn- and do- from asking those we serve.

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Systems of healthcare should begin with the "needs, wishes and values" of those they serve. In veterinary medicine, such an approach places client expectations at the center of service provision. It necessitates the imperative to ascertain the expectations of clients and then institute best practices to meet and exceed those expectations. Evidence-based innovation in service delivery can have profound implications for the quality of care clients receive, and as such, their quality of life when caring for an ill pet. Moreover, quality of care is directly associated with client satisfaction, which, extrapolating from human medicine, has significant implications for increased compliance with medical recommendations, greater client retention, lower rates of malpractice suits, greater profitability, and increased client referrals, making it immediately apparent that attending to client expectations in veterinary medicine is in everyone's best interests: the patient, the client, and the healthcare service. A qualitative interview-based study was conducted to identify the expectations of clients accessing oncology care services at a tertiary referral centre for dogs diagnosed with life-threatening cancer. This study involved 30 in-person single and dyadic audio-recorded interviews with 43 pet owners, using an interview guide with standardized open-ended questions. According to thematic analysis of the transcribed data, six client expectations were identified, namely, (1) information *as an expectation of the consultation*; (2) quick scheduling of the referral and timely service, (3) compassionate service, and (4) continuity of staff and service protocols *as expectations of the healthcare process*; and (5) maintaining quality of life and (6) achieving the goals of treatment *as expectations of the medical intervention*. Based on the findings of this study, a number of recommendations for client service provision may be offered to advance the quality of care that oncology clients receive.

21)

Feline pulmonary carcinoma presenting with limb paresis: Three cases and a review of the literature.

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Limb paresis in association with primary lung neoplasia in cats is a rare finding. There are a total of 9 cases described in recent literature. In humans, arterial tumor embolism is a well-recognized risk for patients with pulmonary carcinoma and can be the presenting sign of occult lung cancer. In addition to reviewing comparative oncology literature on this subject, we describe three new cases that presented to our hospital with sudden onset of limb paresis as a result of arterial thromboembolism in the presence of a primary pulmonary carcinoma and the absence of cardiac disease. All three cats presented with paresis of a single limb, with a cold distal extremity and absence of peripheral arterial pulses. Cardiac evaluation was unremarkable and thoracic imaging revealed a solitary lung mass. Lung lobectomy yielded a survival of 0, 8 and 16 days. Pulmonary carcinoma with intra-arterial tumor emboli was confirmed on histopathology.
Based upon the cases found in literature and the three new cases described in this article, median survival time for cats with paresis in association with pulmonary carcinoma starting from initial presentation was found to be 18 days versus a mean survival time of 6.5 days after surgical intervention. Tumor emboli due to pulmonary carcinoma in cats may cause lameness due to subungal and digital metastasis, bone metastasis, muscle metastasis and paresis due to arterial tumor thromboembolism, as described in our cases and in the reviewed veterinary and human literature. Arterial tumor thromboembolization due to pulmonary carcinoma should be considered as a differential diagnosis in cases of lameness or paresis in older cats. The prognosis in these cases was universally poor. Whereas small pulmonary carcinoma emboli lodge in digits and muscle causing lung-digit syndrome metastasis, larger tumor emboli may obstruct the larger peripheral arteries with acute and devastating effects.

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22)

Surgical Treatment of Canine Splenic Lymphoma: A VSSO Retrospective Study.

L. van Stee[1], S. Boston*[1,2], T. Scase[3], A. Singh[2], G. Romanelli[4], A. Rubio-Guzman[5]; [1]Animal Cancer Centre, [2]Clinical Studies, University of Guelph; [3]Bridge Pathology Ltd, Bristol, UK; [4]VSSO; [5]University of Ghent, Belgium

Lymphoma is generally treated with systemic chemotherapy. It is controversial whether or not some dogs that present with splenomegaly due to lymphoma would benefit from cytoreductive surgery prior to initiating chemotherapy. This study aimed to further characterize and evaluate outcome of canine splenic lymphoma treated with splenectomy. Canine splenic lymphoma treated with splenectomy will have an indolent course of disease overall. Histological classification, treatment with adjunctive chemotherapy and stage will be predictive of survival. Cases were accrued over the VSSO list serve and were included when splenectomy was performed between January 1995 and February 2011, and data on signalment, presenting complaint, reason for surgical intervention, histological diagnosis preoperative treatment and staging performed, post operative treatment, disease free interval, survival time and cause of death were available. Prognostic factors were evaluated with multivariate analysis and included:

age, sex, breed, presenting complaint, stage, adjunctive chemotherapy, histological classification and duration of first remission. Overall median survival was 431 days. B cell splenic lymphoma was associated with a median survival time of 377 days versus 9 days in T cell lymphoma. Age, sex ,weight, hemoabdomen, peripheral lymph node involvement and adjuvant chemotherapy did not significantly affect DFI or MST. Splenic lymphoma without involvement of other organs was associated with a longer MST (532 days) compared to dogs with other organs involved (142 days). Splenic lymphoma without involvement of other organs is correlated with a long survival time. Chemotherapy may not be indicated in these cases. Splenectomy is the treatment of choice for splenic lymphoma.

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23)

Experimental transmission of enzootic nasal adenocarcinoma in sheep.

Scott R. Walsh, Darrick L. Yu, Andrés Diaz-Mendez, Sarah K. Wootton*; Pathobiology, University of Guelph

Enzootic nasal adenocarcinoma (ENA) is a neoplastic disease of sheep characterized by transformation of the ethmoid turbinate. ENA is known to be transmissible but this has not been previously shown experimentally. Enzootic nasal tumor virus (ENTV) is a betaretrovirus of sheep and is thought to be the causative agent of ENA. Confirmation of the role of ENTV in the pathogenesis of ENA has yet to be resolved due to our inability to propagate the virus in cell culture and the lack of an infectious molecular clone. To address the role of ENTV-1 infection in the development of ENA, two week old lambs were infected via nebulization with clarified nasal tumor homogenate from ENA affected sheep. Genomic DNA was extracted from the PBMCs of infected lambs and was screened for the presence of provirus by hemi-nested PCR (hnPCR). Screening failed to detect provirus in all lambs at every point analyzed. At 3 months post infection, clinical symptoms of ENA were observed in one lamb. A multi-focal nasal tumor was identified on a computed tomography scan performed post-mortem and the presence of gross lesions was confirmed upon necropsy. Immunohistochemistry showed the presence of ovine betaretroviral antigens in nasal tumor cells but absence of these antigens in all other tissues tested. HnPCR screening of genomic DNA for the provirus was positive for all tissues, excluding the trachea and the lymph nodes. This is the first report of experimental induction of ENA in sheep.

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24)

Distinct Regulatory Influences of the ShcD Phosphotyrosine Adaptor Protein on EGF Receptor Signaling.

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Members of the Shc protein family function as signal transduction adaptors to couple activated receptors with proximal effectors. They are distinguished by their CH2-PTB-CH1-SH2 modular design, and are best characterized as mediators of Ras / MAPK signaling downstream of the

Epidermal Growth Factor (EGF) Receptor Tyrosine Kinase, an important prognostic marker in cancer. The recent discovery of the fourth homolog, ShcD, has prompted its characterization. We now report that ShcD binds via its PTB domain to the phosphorylated EGFR residue Y1148 in response to EGF stimulation. Under these conditions, ShcD itself becomes tyrosine phosphorylated and inducibly binds Grb2. CLSM further reveals extensive colocalization of ShcD and EGFR in punctae distributed throughout the cell. Notably, we find a constitutive increase in EGFR –Y1068 phosphorylation in the presence of ShcD, which can be abolished through targeted mutation of its PTB domain. Receptor hyperphosphorylation appears to be a unique consequence of ShcD activity, as it is not facilitated by the ShcA homolog. While EGF response in non-transfected cells is marked by ligand internalization, perinuclear trafficking of the receptor, MAPK activation, and eventual attenuation of phospho-specific signaling, cells expressing ShcD demonstrate none of these features. We have thus identified novel phosphorylation, trafficking, and signaling characteristics of ShcD in the context of the EGF receptor, which may have important implications in the development and progression of diseases such as cancer.

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25)

Continuous low-dose (metronomic) oral chemotherapy therapy of canine appendicular osteosarcoma.

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Osteosarcoma is the most common primary bone tumour in dogs with most dogs dying of metastatic disease despite surgery and maximum tolerated dose (MTD) adjuvant chemotherapy. In veterinary oncology, continuous low-dose (metronomic) schedules of chemotherapy have become more popular for patient management. Therefore, the purpose of this study was to retrospectively investigate the effectiveness of the addition of low-dose metronomic chemotherapy following curative-intent amputation and adjuvant carboplatinum chemotherapy to dogs with appendicular osteosarcoma. Materials & Methods: Dogs presented to the Ontario Veterinary College with a diagnosis of appendicular osteosarcoma who underwent limb amputation followed by adjuvant carboplatinum chemotherapy were eligible. Dogs completed the amputation and carboplatinum and either received no further treatment (control); or continued with a metronomic chemotherapy protocol (treated) consisting of cyclophosphamide (10-25 mg/m^2 q 24-48h); nonsteroidal anti-inflammatory (NSAID); and doxycycline (5 mg/kg q24h). Results: Sixty-one dogs entered the study and 21 dogs were evaluable. The control group had 13 dogs with a median metastasis-free interval of 178 days and a median survival time of 504 days. The treatment group had 8 dogs with a median metastasis-free interval of 332 days (P=0.16) and a median survival time of 340 days (P=0.55). Conclusion: Low-dose chemotherapy had few adverse effects; however, efficacy was difficult to determine perhaps due to lack of prospective randomization of the dogs into the groups. Future studies are needed to determine selection of metronomic agents (timing and dosing schedules); biomarkers for efficacy and toxicity; and combinations with standard drug dosing, newer targeted drugs, or immunotherapy.

26)

Preventative effects and Darkening and Non-darkening Cranberry Beans on Biomarkers of Colonic Inflammation in Mice.

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Whole beans consumption is associated with a reduced risk of colon cancer. Inflammatory bowel disease (IBD) is a major risk factor for colitis-associated colon cancer. Beans are rich in phenolics with known anti-inflammatory and anti-oxidative properties. This study will examine the preventative effects of consuming whole beans, with different levels of phenolics, on colonic inflammation in mice. It is hypothesized that phenolic-rich beans will reduce colonic inflammation to a greater extent than phenolic-poor beans. C57BL/6 male mice were fed a basal diet (BD) supplemented with or without 20% cooked darkening and non-darkening cranberry bean flour for 21 days. On day 21, mice fed bean diets were given BD for the remainder of the study and colitis was induced by exposing mice to 2% dextran sulphate sodium (DSS) in their drinking water for 7 days. In mice fed BD only, exposure to DSS caused a reduction in water and diet intake, increased weight loss, stool consistency and stool blood scores, and increased colon weight. These outcomes were all attenuated in mice that were fed cranberry bean diets prior to DSS exposure. In addition, bean diets did not suppress Myeloperoxidase levels compare to DSS+BD only group. Interestingly, non-darkening cranberry bean had a greater effect on preventing diarrhea than darking cranberry bean diets. The total phenolic, flavonoid and anthocyanin content, and antioxidant activity were higher in the darkening cranberry beans compared to non-darkening cranberry beans. Overall, this study demonstrates the preventative role of beans on DSS-induced colitis. However, phenolic rich bean did not give added benefit compare to phenolic poor beans suggesting that total phenolic levels and antixodiant activity may not be as important as hypothesized. Other bioactives in bean may also play a role in colitis prevention. Future studies are need to investigate bean phenolic and other bioactives in colitis.

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27)

Characterization of endothelial-mesenchymal-transition (EndoMT) markers in response to transforming growth factor-beta (TGFβ) in bovine aortic endothelial cells (BAEC).

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Angiogenesis, the formation of new blood vessels by sprouting from pre-existing ones, is required for tumor growth and metastasis. Transforming growth factor-beta (TGFβ) promotes angiogenesis and is also known to induce endothelial-mesenchymal-transition (EndoMT), a phenomenon of cellular plasticity by which endothelial cells become fibroblastic and motile. We hypothesize that TGFβ-induced EndoMT enables endothelial cells to detach from the blood vessel lining and migrate to form the sprout that gives origin to a new vessel during angiogenesis. This study characterized EndoMT in response to TGFβ, and TGFβ plus vascular endothelial growth factor (VEGF), an angiogenic factor known to cooperate with TGFβ in promoting angiogenesis. For this purpose, bovine aortic endothelial cells (BAEC) were stimulated with varying concentrations of TGFβ (0.1-5 ng/mL) plus VEGF (1-50 ng/mL) for 48,

96 and 144 hours. Confocal imaging and immunoblotting analyses revealed the strongest EndoMT response at 5 ng/mL of TGFβ and after 144 hours of exposure, which was visualized by loss of the tight junction marker ZO-1 and the adherens junction marker VE-cadherin, in addition to induction of Snail, an EndoMT-inducing transcription factor. VEGF does not significantly potentiate TGFβ's effects. These results suggest that EndoMT induction in BAECs requires high concentrations and prolonged exposure to TGFβ and is not significantly influenced by VEGF. We are currently investigating the potential relationship between EndoMT and blood vessel sprouting using in vitro models of angiogenesis. Our findings will help define TGFβ's role in angiogenesis and its potential use as a target for anti-angiogenic cancer therapy.