OVC | Institute for Comparative Cancer Investigation | University of Guelph

Program

Cancer Research Symposium

Thursday, June 3, 2010
Room 1714 LLC, Ontario Veterinary College
University of Guelph

Introductory Remarks

Welcome to the 3rd 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 biological elements, to clinical therapies and on to social, emotional and even philosophical aspects of this often-devastating disease. 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. Matthew Breen, who is this year's Arthur Willis Distinguished Speaker. We hope you find this symposium interesting and informative, and that it leads to fruitful research collaborations for all our attendees.

Co-Organizers
Brenda Coomber and Jon LaMarre.
Biomedical Sciences, University of Guelph

Additional thanks to Ms Barb Gaudette, OVC Office of the Dean, for her administrative expertise and invaluable assistance in organizing this event, and to Adrian Hollingbury and his crew at the OVC Dining Hall for their hospitality service.

University of Guelph Institute for Comparative Cancer Investigation

Cancer Research Symposium Schedule

June 3, 2010 Room 1714 OVC LLC

9:00 Welcome: Dr. Gordon Kirby, Associate Dean, OVC

9:05 Introductory Remarks: Dr. Jon LaMarre, Professor, Biomedical Sciences, OVC

Session 1: Molecular Aspects of Cancer Biology

9:15 Dr. Brenda Coomber, Professor, Biomedical Sciences, OVC
"The impact of tumor ischemia on cancer progression and response to therapy"

9:40 Dr. Ray Lu, Associate Professor, Molecular and Cellular Biology, CBS
"A CREBZF/Zhangfei isoform activates CHOP and promotes apoptosis during prolonged endoplasmic reticulum stress"

10:05 Lisa Kellenberger, Ph.D. Candidate, Biomedical Sciences, OVC – *Short Presentation: "Hyperglycemia accelerates the progression of epithelial ovarian cancer and anti-hyperglycemic drugs offer novel treatment opportunities"*

10:20 – 10:40 Coffee Break Room 1707 B & C

10:40 Dr. James Kirkland, Associate Professor, Human Health and Nutritional Sciences, CBS *"The impact of niacin status on chemotherapy-induced myelosuppression and leukemogenesis: a rat model"*

11:05 Dr. Marc Coppolino, Associate Professor, Molecular and Cellular Biology, CBS
"Trafficking and Secretion of Matrix Metalloproteinases during Tumour Cell Invasion"

11:30 Breanne Anderson, M.Sc. Candidate, Human Health and Nutritional Sciences, CBS - *Short Presentation: "N-3 PUFA exposure by diet or genetics early in life modifies mammary gland development and fatty acid composition in FVB mice"*

11:45-1:40 Lunch (provided) and Poster Session, Room 1707 B & C.
Poster presenters please attend your posters between 12:15 and 1:30

Session II: The Patient and Their Disease

1:40 Introductory Remarks: Dr. Brenda Coomber, Professor, Biomedical Sciences, OVC

1:50 Dr. Anthony Abrams-Ogg, Associate Professor, Department of Clinical Studies, OVC *"Large-field radiation therapy for the treatment of canine lymphoma"*

2:15 Michelle Oblak, DVSc. Candidate, Clincial Studies, OVC – *Short presentation: "MST in dogs having palliative RT for appendicular OSA"*

2:30 Amandine Lejune, Oncology Intern, OVC – *Short Presentation: "A retrospective study of canine thyroid carcinoma: evaluation of the significance of histological subtype and other factors on prognosis"*

2:45 Dr. Gordon Kirby, Professor, Department of Biomedical Sciences, OVC *"Development of a diagnostic test for canine hemangiosarcoma: identification of serum biomarkers"*

3:10 Melanie K.B. Wills, Ph.D. Candidate, Molecular and Cellular Biology, CBS – *Short Presentation: "Teaching an Old Dogma New Tricks: The Enigmatic Role of Shc Adaptor Proteins in Cancer"*

3:25 Lisa Wenger, PhD Candidate, Department of Family Relations and Nutrition, CSAHS - *Short Presentation: "Seeking understanding: A grounded theory study of how men with cancer experience help-seeking"*

3:50 - 4:00 Break

4:00 Keynote Address:

"The Domestic Dog- A Genome with Two Tales"

Dr. Matthew Breen

Professor of Genomics
Molecular Biomedical Sciences
College of Veterinary Medicine
North Carolina State University

5:00 Closing Reception
Room 1707 B & C
Cash Bar

ABSTRACTS

"The Domestic Dog – a Genome with Two Tales"

Matthew Breen, PhD CBiol FSB
Professor of Genomics, Molecular Biomedical Sciences
College of Veterinary Medicine
North Carolina State University

Abstract: The application of genomics to canine biomedical research has resulted in significant advances as we strive to enhance the health and welfare of our companions. Over the past several years we have recruited tumor tissues and blood samples from several hundred dogs presenting with a variety of cancers. At the same time we have generated a series of sophisticated molecular cytogenetic reagents and resources that complete the genomics "toolbox", providing a means to interrogate tumor specimens for genomic organizational changes. We have demonstrated the presence of numerous cytogenetic signatures associated with cancer subtypes and that also are evolutionarily conserved with the corresponding cancers in humans. These data provide strong evidence for a shared pathogenetic origin of several cancers affecting both human and dog. For instance,we have identified genetic changes in canine lymphoma that are associated significantly with response to routine chemotherapy and that allow prediction of survival time. An overview of these studies will be presented.

Biographical Sketch: Dr. Matthew Breen is Professor of Genomics at the North Carolina State University's College of Veterinary Medicine in Raleigh, NC. A graduate of the University of Liverpool, Dr. Breen continued his postgraduate work with the Medical Research Council, U.K. where he developed improved laboratory techniques for the human genome mapping project, followed by four years as Head of the DNA group for the Australian Equine Blood Typing Research Laboratory in Queensland. Returning to the UK, Breen's laboratory then developed molecular cytogenetics reagents, resources and techniques for application to canine and equine genome mapping, comparative cytogenetics and cancer studies. In 2002, he moved to North Carolina State University, where his research interests continue to focus on genomics, genome mapping and the comparative aspects of canine cancer. His work is funded by the Morris Animal Foundation, the American Kennel Club and the USA National Institutes of Health. He has published over 100 peer reviewed scientific articles and has served on several scientific advisory committees associated with canine cancer, and is sought after as a scientific reviewer for numerous funding agencies and scholarly publications.

Dr. Breen is this year's Arthur Willis Distinguished Speaker

Abstracts for Oral Presentations

The impact of tumor ischemia on cancer progression and response to therapy
Brenda Coomber, Biomedical Sciences, University of Guelph

Solid tumors are heterogeneous structures, containing regions of healthy, well-perfused tissue adjacent to areas of ischemia. It has long been known that the stressful microenvironment found in ischemic tissues may lead to tumor progression, and our laboratory has been exploring this issue using in vitro and xenograft models of cancer. Exposing human cancer cells to ischemic conditions (low oxygen, low glucose, or a combination) in vitro results in defective DNA repair and generation of de novo mutations, altered regulation of gene expression, and changes in cancer cell signaling, all of which could contribute to cancer progression. Anti-angiogenic therapy directed at reducing blood vessel growth in colorectal cancer xenografted tumors paradoxically lead to increased *KRAS* oncogene activation and enhanced tumor growth. *KRAS* mutations arose preferentially in ischemic regions of these tumors, and ischemia was enhanced by angiogenesis inhibition. As well, the putative apoptosis inducing agent dichloroacetate (DCA) failed to induce apoptosis in all cancer cells examined, and more importantly, provided a cytoprotective effect under ischemia. DCA also induced significantly increased growth of colorectal cancer xenografts, and decreased apoptosis of cancer cells in ischemic but not well oxygenated regions of treated tumors. These studies demonstrate the important role played by the microenvironment within solid tumors, and highlight its potential for interaction with anti-cancer agents to generate unexpected outcomes and potential disease progression.

A CREBZF/Zhangfei isoform activates CHOP and promotes apoptosis during prolonged endoplasmic reticulum stress
Ray Lu, Molecular and Cellular Biology, University of Guelph

Stress in the endoplasmic reticulum (ER) triggers a well-orchestrated cellular response, the so-called unfolded protein response (UPR). There is increasing evidence that the UPR is important for the development and growth of tumors (such as solid tumors), which proliferate under hypoxia or glucose deprivation conditions known to activate the UPR. The cellular transcription factor CREBZF (Zhangfei or ZF) was identified by its potential association with Herpes Simplex Virus-1 replication, and has been implicated in cellular stress responses through its interaction with other stress response proteins, such as ATF4. Here we investigated the production of four CREBZF isoforms, which arise from translational initiation of a downstream AUG at codon 83 and mRNA alternative splicing that adds an IFFFR pentapeptidyl tail to the C-terminus. We found that in addition to transcriptional induction, the short-tailed CREBZF (stZF) isoform was specifically induced by prolonged ER stress treatment and amino acid deprivation. This stZF isoform is a potent transcriptional activator of the pro-apoptotic protein CHOP. Overexpression of stZF activates transcription of CHOP through a CCAAT enhancer binding protein (C/EBP)-ATF site, and promotes apoptosis. We propose that 1) CREBZF is a key component of the integrated stress response; 2) stZF is tightly regulated and induced primarily under prolonged cellular stress, and is essential for the role of CREBZF in inducing CHOP and promoting cell death.

Hyperglycemia accelerates the progression of epithelial ovarian cancer and anti-hyperglycemic drugs offer novel treatment opportunities

Lisa Kellenberger[1], Alison Holloway[2], Hertzel Gerstein[3], and Jim Petrik[1] [1]Biomedical Sciences, University of Guelph, Guelph, ON, Canada; [2] Department of Obstetrics and Gynaecology, McMaster University, [3]Division of Endocrinology and Metabolism, McMaster University, Hamilton

Circulating glucose is a potential source of metabolic fuel for transformed cells and therefore may be an important mediator of epithelial ovarian cancer (EOC). Epidemiological studies comparing dysglycemia and cancer development provide intriguing preliminary support for this idea. Hyperglycemia is also associated with increased angiogenesis in many tissues. We hypothesize that hyperglycemia will accelerate the growth of ovarian tumours by both increasing the availability of metabolic fuel and by stimulating tumour angiogenesis. We have found that in a Type I diabetic mouse model, even short-term exposure to hyperglycemia (10 days) can profoundly accelerate the growth of ovarian tumours. *In vitro*, hyperglycemic conditions increase the metabolic viability of human (SKOV-3) and murine (ID8) EOC cell lines, as well as murine endothelial cells, while normal human ovarian surface epithelium is unaffected by hyperglycemia. In addition, the anti-diabetic compounds metformin and rosiglitazone directly reduce EOC cell viability in culture independent of their *in vivo* glucose lowering effects. Preliminary data suggest that these drugs also reduce expression of angiogenic factors. Taken together, these results show that EOC progression is accelerated in the presence of hyperglycemia and that use of anti-hyperglycemic drugs may be a novel treatment option targeting both tumour cells and tumour microvasculature.

The impact of niacin status on chemotherapy-induced myelosuppression and leukemogenesis: a rat model

James Kirkland, Human Health and Nutritional Sciences, University of Guelph

Niacin is required for the formation of NAD, which is used as a substrate in a wide variety of ADP-ribosylation reactions. Many of these reactions are involved in the maintenance of genomic stability, and the sun sensitivity of pellagra is an illustration of the failure of DNA repair during niacin deficiency. We have examined the impact of niacin status in a rat model on the response to alkylating agents. Niacin deficiency during drug exposure enhances acute myelosuppression and the long term development of leukemias. This is associated with large changes in poly(ADP-ribose) formation, disruption of excision repair of DNA, and dramatically increased genomic instability. The talk will end with a theoretical discussion of the additional potential of high niacin status to improve tumor cell killing via the AIF pathway.

Trafficking and Secretion of Matrix Metalloproteinases during Tumour Cell Invasion

Marc Coppolino, Molecular and Cellular Biology, University of Guelph

Following neoplastic transformation, tumor cell invasion of the ECM is required for the growth of primary tumors and for the ability of tumors to metastasize. Invasion through an

ECM barrier is a complex, step-wise process involving cell adhesion, ECM proteolysis by secreted matrix metalloproteinases (MMPs), and migration of the tumor cell. The intracellular mechanisms that regulate these activities are subjects of intensive study and evidence now suggests that regulated trafficking and secretion of MMPs is central to the control of ECM degradation and cellular invasion. The goal of the current studies is to define the mechanisms by which SNARE-mediated membrane traffic regulates cell invasion. (SNARE: soluble NSF attachment protein receptor, NSF: N-ethylmaleimide-sensitive factor.) SNAREs are direct regulators of membrane fusion and they are central factors within all intracellular membrane trafficking pathways. Recent studies from our laboratory have identified a role for SNARE-mediated traffic in tumour cell invasion. We have determined that inhibiting the function of SNAREs (SNAP23, VAMP3 or syntaxin13), using inhibitory SNARE constructs or RNA interference, impairs secretion of MMPs. This causes reduced degradation of ECM, impairs the formation of invadopodia and impedes cell invasion in HT-1080 fibrosarcoma cells. We propose a model of cell invasion in which SNARE-mediated membrane traffic regulates the intracellular transport and targeted secretion of MMPs, thus facilitating tumour cell movement. The increased understanding of cell invasion provided by these studies allows insight into the biological mechanisms of cancer progression and the development of metastatic forms of the disease.

N-3 PUFA exposure by diet or genetics early in life modifies mammary gland development and fatty acid composition in FVB mice

Anderson, Breanne M; Hillyer, Lyn; Maclennan, Mira; Kang, Jing X; Ma, David WL. Human Health and Nutritional Sciences, University of Guelph

Breast cancer is the most common form of cancer among Canadian women and the second leading cause of cancer death in this population. It is currently unknown the extent to which dietary modifications during early life can affect mammary gland development, however it has been estimated that 35% of breast cancers could be prevented by appropriate dietary alterations. In particular, n-3 polyunsaturated fatty acids (PUFA) found in marine oils have demonstrated the ability to reduce mammary epithelial stem cell populations susceptible to carcinogens in rodents, however these findings were observed with very high n-3 PUFA intakes. In the present study, a combined dietary and genetic approach was used to assess the effects of n-3 PUFA on mammary gland development and fatty acid composition at physiologically relevant doses. Wild-type mice fed n-6 PUFA were compared to Fat-1 transgenic littermates, capable of synthesizing n-3 PUFA from n-6 PUFA. Additional wild-type mice were fed n-3 PUFA and compared to Fat-1 transgenic littermates fed the same diet. Mice were sacrificed at 3, 4, 5, 6, 7, 9 and 12 weeks of age. Pubertal onset was monitored from 3 to 5 weeks of age. Mammary gland development was assessed by wholemount and enumeration of terminal end bud structures. Mammary gland fatty acid composition was determined by gas liquid chromatography. Pubertal onset was delayed in both dietary and genetic groups with high n-3 PUFA status ($p < 0.05$). At 3 and 4 weeks of age, n-3 PUFA from diet but not genetics alone yielded significant changes in mammary gland development ($p < 0.05$). Both diet and genetics had a significant influence on mammary gland fatty acid composition as early as 3 weeks of age, through to 12 weeks post-natal, however n-3 PUFA by diet resulted in significantly greater incorporation of n-3 PUFA than by genetics alone.

The significance of these findings suggests that early life dietary modification by differential levels of n-3 PUFA affects mammary gland development of FVB mice, particularly at 3 weeks of age, before the onset of puberty.

Large-field radiation therapy for the treatment of canine lymphoma
Anthony Abrams-Ogg, Department of Clinical Studies, University of Guelph

Large-field radiation therapy includes total body irradiation (TBI), half-body irradiation (HBI), and local irradiation using larger fields, and higher doses and/or fewer fractions, than in conventional therapy. All modalities may be used for palliation, while TBI and HBI may also be used with curative intent. TBI is most often used in preparation for hematopoietic stem cell transplantation. This is a standard treatment for humans, but, although pioneering work was done on dogs, it is rarely used by veterinarians. Low-dose TBI, and HBI, have been used for relapsed lymphoma, with results comparable to those of chemotherapy protocols. The main value of HBI appears to be as consolidation therapy following chemotherapy-induced complete remission. Results of studies at the University of Guelph and other veterinary hospitals have shown that HBI as consolidation therapy achieves results equivalent or superior to those achieved with conventional consolation and/or maintenance chemotherapy. However, improved results have not be sufficiently dramatic to establish HBI as a standard first-line treatment in veterinary oncology, and future studies should be directed at identifying which subtypes, if any, are most likely to benefit from one treatment versus another. Local irradiation has been used successfully to palliate local signs in dogs failing systemic chemotherapy.

MST in dogs having palliative RT for appendicular OSA. *Michelle Oblak, Sarah Boston, Steve Patten, Geri Higginson, Gabrielle Monteith Department of Clinical Studies, University of Guelph.*

Osteosarcoma (OSA) is the most common primary bone tumor in dogs. When administering palliative radiation therapy for dogs with OSA, a two to four fraction 8-10 Gy dose is used. In addition, pamidronate and chemotherapy are recommended to help prolong survival. The purpose of this study was to assess survival time in dogs that underwent palliative radiation therapy (RT) for appendicular OSA from 1989-2009 and evaluate factors affecting survival. Our hypothesis was that dogs who receive RT, chemotherapy and pamidronate would have the longest survival times and other parameters would be similar to previously reported. All dogs that underwent palliative RT for appendicular OSA during this time frame were included. Any dogs that did not have a known euthanasia date, or if follow up was lost within 120 days of their initial visit were censored for the purpose of survival analysis. Cox proportional hazard models and Kaplan Meier survival functions were used. A p value of <0.05 was considered significant. Seventy dogs met the inclusion criteria, twenty were censored for incomplete survival data. Survival time was longest for dogs receiving RT and chemotherapy (307 days; 95%CI= 279-831) and shortest in dogs receiving RT and pamidronate (69 days; 95%CI=47-112 days). The difference in survival between dogs who

received pamidronate and those who did not in this population was statistically significant in a univariate ($p=0.0385$) and multivariate analysis ($p=0.0015$). The addition of chemotherapy improved survival ($p<0.001$). Based on this study, chemotherapy should be recommended in addition to palliative RT to improve survival. Further evaluation of pamidronate is required before making it part of a standard protocol.

A retrospective study of canine thyroid carcinoma: evaluation of the significance of histological subtype and other factors on prognosis

Amandine Lejeune[1], Sarah Boston[1], Galina Hayes[2], Robert A. Foster[3]; [1]Department of Clinical Studies; [2]Department of Population Medicine; [3]Department of Pathobiology, University of Guelph

Introduction: Thyroid carcinomas in dogs have a high rate of metastasis. The histological cell type in dogs is thought to be unrelated to prognosis.
Material and method: 62 records of dogs with thyroid carcinoma were examined. Total thyroxin (T4) level, coagulation profile, stage, immunohistochemistry were collected and analyzed. Potential prognostic variables affecting survival were identified. Survival times based on the histological subtype of thyroid carcinoma and treatment modality were compared.
Results: Thyroid carcinoma was more common in older large breed dogs. Eight dogs had evidence of gross metastasis at the time of diagnosis. Presence of metastasis was associated with reduced survival time. Tumor size was significantly associated with survival time. Immunohistochemical and histological variables were not significantly associated with survival time. Total T4 was identified as a strong independent predictor of survival hazard. Inclusion of surgery in the treatment protocol was associated with a significant improvement of outcome and survival time.
Conclusions: Inclusion of surgery in the treatment protocol results in significant improvement of survival time and outcome. Total thyroxin levels may be useful to predict prognosis, although further investigation is warranted to support this finding. Immunohistochemistry did not help to differentiate dogs at risk for shorter survival times.

Development of a diagnostic test for canine hemangiosarcoma: identification of serum biomarkers

Gordon Kirby, Department of Biomedical Sciences, University of Guelph

Canine hemangiosarcoma (HSA) is a common malignant neoplasm derived from vascular endothelial cells with characteristic early, aggressive metastasis. Diagnosis of HSA is challenging due to the lack of sensitive and specific tests for early detection. We hypothesized that specific proteins that are increased in serum of dogs with HSA might represent useful biomarkers of the disease. The serum proteome of dogs with HSA was compared to that of control dogs from the Ontario Veterinary College Teaching Hospital. Two-dimensional difference gel electrophoresis revealed increased levels of a protein subsequently identified by liquid chromatography and tandem mass spectrometry as the lipocalin region of collagen XXVII alpha 1, the product of the *COL27A1* gene. Western blot

analysis showed that levels of the collagen XXVII peptide in serum of 34 dogs with large metastatic HSA burdens were, on average, 5.5 fold higher than in 43 controls. While serum collagen XXVII peptide levels for dogs with osteosarcomas, lymphomas, carcinomas and inflammatory disease were also elevated, values were significantly more variable. Receiver operating characteristic curves revealed an estimated area under the curve of 83% for HSA cases whereas areas for other neoplastic and non-neoplastic diseases were non-discriminatory. Finally, serum collagen XXVII peptide levels were markedly reduced in dogs following tumor removal by splenectomy and chemotherapy but increased with tumor recurrence. These results indicate that collagen XXVII peptide is a potentially useful biomarker for HSA diagnosis at late stages of disease progression. The positive predictive value of this diagnostic test awaits further validation.

Teaching an Old Dogma New Tricks: The Enigmatic Role of Shc Adaptor Proteins in Cancer

Melanie K. B. Wills and Nina Jones, Cellular and Molecular Biology, University of Guelph

Proteins of the Shc family function as molecular adaptors in cellular signal transduction cascades by coupling stimulated membrane receptors to downstream effectors. The Shc homologs thus influence activation of major cellular pathways that elicit survival, proliferation, migration, and differentiation. Under homeostasis, this promotes organismal development and maintenance, however in disease it can establish or potentiate the pathology by facilitating aberrant signaling. Indeed, Shc adaptors are associated with a variety of cancers, including those of the breast, skin, brain, and neuroendocrine systems. Decades of Shc research have revealed two surprising observations: 1) the homologs are distinct from one another in their oncogenic potential, and 2) the tumorigenic capacity, and mechanism, of a single Shc homolog is highly context dependent. Recently, the simultaneous discovery of the existence and metastatic property of the fourth family member, ShcD, has prompted its molecular characterization. Preliminary findings from qRT PCR performed on human biopsy specimens suggest that ShcD is considerably upregulated in brain cancers, while cell-based assays reveal a non-canonical role for ShcD as a repressor of Erk phosphorylation downstream of the Ret receptor. Reconciling these molecular characteristics with the oncogenic progression of the tumours will further define the role of signaling adaptors in cancer.

Seeking understanding: A grounded theory study of how men with cancer experience help-seeking

Lisa Wenger, Department of Family Relations and Nutrition, University of Guelph

A growing body of literature has organized around the understanding that limited or delayed help-seeking can function as a key risk to men's health. As practitioners struggle to engage men in health services, the need for research and policy addressing men's help-seeking is relevant to individuals facing illness, their networks of family and friends, and the formal systems seeking to support them. The current literature offers important insights into *why* men might delay or avoid engaging with health professionals when facing troubling signs

and symptoms, but there remain key gaps in our understanding. Among these, little attention has been devoted to *how* men experience help-seeking. To address this, my research is oriented around two central research questions: (a) how are men with cancer perceiving, interpreting, and understanding needs and support between the time when they first sense something is wrong and when they enter treatment? and (b) how are they building patterns of support (informal and formal) with others during this time? Over the course of this study I will interview men who have recently entered treatment for any form of cancer and my research process is organized by a grounded theory methodology that seeks to find patterns across the individual stories men tell. With theory oriented toward practical purposes, I believe the information gathered could enable the capacity of health services to engage men in timely use of effective supports, to inform what services are offered, and influence how they are designed and delivered.

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Abstracts for Poster Presentations

1) Naked1 Antagonizes Canonical Wnt Signaling by Preventing Nuclear Accumulation of β-catenin.

Terry Van Raay[1], Haiting Ma[2], Nicholas J. Fortino[1], Cunxi Li[3], Jeffrey L. Franklin[3], Lilianna Solnica-Krezel[2], Robert J. Coffey[3] [1]University of Guelph, Guelph, [2]Vanderbilt University, Nashville, (TN) [3]Vanderbilt Medical Center, Nashville, TN

Cyto-nuclear shuttling of β-catenin is at the epicenter of the canonical Wnt pathway and is the driving force behind the initiation of many cancers. However, the mechanisms that regulate this process in both development and disease are poorly understood. In particular, how Wnt-induced negative regulators impact nuclear entry of β-catenin has not been studied. Recently, one consistent and conserved target of the canonical Wnt pathway, Naked Cuticle (Nkd) in Drosophila or Naked1 (Nkd1) in vertebrates, has been identified as a Wnt induced negative regulator of canonical Wnt signaling. Genetic epistasis studies in Drosophila have demonstrated that Nkd functions somewhere between Disheveled (Dvl) and β-catenin, however, the mechanism of Nkd antagonism is unclear. To address the molecular nature of Nkd1 function, we utilized the multipotent precursors of the early zebrafish blastula as an in vivo cell model of Wnt regulation. Prior to gastrulation, the zebrafish blastula consists of multiptotent, embryonic cells that are highly responsive to Wnt signaling. Using standard biochemical and cellular techniques we demonstrate that Nkd1 is acting at the level of nuclear import of β-catenin. Further, we argue that Nkd1 activity is dependent on active Wnt signaling. Our results suggest a highly conserved mechanism of Wnt feedback inhibition.

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2) Down-regulation of GSTA1 by EGF is required for Caco-2 cell proliferation.

Kelly O'Rourke and Gordon M. Kirby. Biomedical Sciences, University of Guelph.

Alpha class glutathione S-transferases (GSTA) play an important cytoprotective role in detoxifying electrophiles and are also inhibitors of c-Jun N-terminal kinase (JNK). We have previously shown that mitogens such as IL-1β and TPA down-regulate GSTA1 expression in Caco-2 cells. While GSTA1 expression progressively increases as Caco-2 cells differentiate post-confluency, the importance of GSTA1 in cellular proliferation is unclear. We tested the hypothesis that down-regulation of GSTA1 by epidermal growth factor (EGF) is required for proliferation of Caco-2 cells. Treatment of Caco-2 cells with EGF (1, 10, 100 ng/ml) for 24 h caused a significant dose-dependent reduction in GSTA1 mRNA levels to a maximum of 25% of control values. In luciferase reporter assays, deletion constructs mapped EGF-mediated repression of GSTA1 transcriptional activity to within a region -355 and -165 bp upstream of the coding region. Site-directed mutatgenesis revealed that this repression was mediated via a HNF-1 site in this region. Real-time RT-PCR showed that treatment with EGF resulted in significant increases in mRNA levels of the proliferation markers cyclin D1 and c-myc by1.5-fold and 2.4-fold, respectively. Finally, proliferative status assessed by measuring MTS absorbance was significantly reduced in EGF-stimulated pre-confluent Caco-2 cells in which GSTA1 was overexpressed by transient transfection with a pcDNA3.1-GSTA1 expression plasmid. Collectively, these findings suggest that low levels of GSTA1

expression are a requirement for Caco-2 cell proliferation but the mechanism by which GSTA1 modulates proliferation is unclear. We are currently investigating whether GSTA1-mediated suppression of Caco-2 cell proliferation involves reduced activation of JNK by EGF. In conclusion, these findings demonstrate a novel role for GSTA1 in modulating proliferative activity of Caco-2 cells. Enhanced understanding of the role of GSTA1 in cellular proliferation may lead to novel insights for therapeutic strategies for various inflammatory and neoplastic diseases.

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3) Molecular Mechanisms Involved in Jaagsiekte Sheep Retrovirus Env-induced Oncogenesis

Nicolle Petrik, Sarah Wootton, Department of Pathobiology, University of Guelph

Jaagsiekte sheep retrovirus (JSRV) is a betaretrovirus that causes ovine pulmonary adenocarcinoma (OPA) in sheep and closely resembles pulmonary adenocarcinoma in humans. JSRV induces lung tumors in sheep through the expression of an oncogenic envelope (Env) protein. 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 JSRV, may have a role in the pathogenesis of lung cancer. JSRV Env is capable of inducing tumors in both sheep and mice, but the role of JSRV in human lung cancer remains unclear. We examined the presence of JSRV Env in multiple types of human lung tumor samples by immunohistochemical staining with an Env-specific monoclonal antibody and by PCR amplification with Env-specific primers. The results from these studies reveal that a subset of human lung cancers express an antigen that cross-reacts with a JSRV Env-specific monoclonal antibody and that JSRV-like Env sequences can be amplified from human lung cancers. To better understand the role that Env may have in lung tumorigenesis, we currently are characterizing a mouse model that expresses the JSRV Env protein in lung tissue using a replication-defective adeno-associated virus vector. Preliminary results reveal that tumors from our mouse model have similar characteristics to both sheep and human adenocarcinomas and therefore could serve as a valuable model to better understand the mechanism of JSRV Env in lung tumorigenesis.

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4) Interplay between TGF-beta and VEGFR2 expression in colorectal cancer blood vessels.

Kuczynski EA, Viloria-Petit AM, Coomber BL, Biomedical Sciences, University of Guelph

Vascular endothelial growth factor signaling is a target for anti-angiogenic cancer therapy. Our group has recently observed heterogeneous expression patterns of VEGF receptor 2 in the vasculature of human and of mouse xenografted tumors. We see a high proportion of blood vessels negative for VEGFR2 expression, particularly in colorectal tumors (40%), but its cause and significance is unknown. Differential activity of transforming growth factor beta (TGF-β) may contribute to this heterogeneous expression pattern. Importantly, TGF-β may down-regulate VEGFR2 in normal endothelium, and is often over-expressed and contributes to the progression of colorectal tumors. Using bovine aortic endothelial cells

(BAEC) we found that TGF-β1 significantly repressed VEGFR2 transcripts and protein up to 89% in a dose-dependent fashion ($p<0.05$). Serum free conditioned media collected from various malignant human colorectal carcinoma cell lines induced down-regulation of VEGFR2 in BAEC, an effect that was blocked by inhibitors of TGF-√ receptor I. This novel finding of vascular heterogeneity in human tumors represents a potential mode of anti-angiogenic therapeutic resistance in cancer.

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5) Retrospective evaluation of factors influencing completeness of excision of skin and subcutaneous mast cell tumors and soft tissue sarcomas in 100 dogs

Beatriz Monteiro, Sarah Boston, Gabrielle Monteith. Department of Clinical Studies, University of Guelph.

The objective of this study was to evaluate possible risk factors for incomplete surgical margins of skin and subcutaneous STS and MCT in dogs. One hundred dogs that had wide excision with curative intent were evaluated for age, sex, breed and weight of dog; tumor type, grade, size and location; histological report of completeness of excision and level of the surgeon's training (ACVS residents and board certified surgeons with (ACVS-SO) and without (ACVS) additional training in surgical oncology). Decreased body weight was a significant risk factor for incomplete margins (p=0.03, odd's ratio= 0.96) as well as increased tumor size (1.4% increase in risk of incomplete excision per $cm^{2;}$ p=0.02). Sex, age, breed, location, grade, tumor type, re-excision and level of surgeons training (p=0.0711) were not significant for completeness of excision. There was significant difference in the risk of an incomplete excision of STS when the tumor was excised by a resident (p=0.0123, odd's ratio= 1.22) when compared to ACVS and ACVS-SO. Sex, breed, age, re-excision, grade, location and type of tumor and level of training of the surgeon are not a risk factor. ACVS residents, increased tumor size and decreased body weight are associated with an increased risk of incomplete excision.

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6) The role of perforin expression in canine cutaneous histiocytoma tumor regression

M. Neta[1], P. Katavolos [2], Josepha DeLay [3,] D. Wood [1], P.F. Moore[4], R.M. Jacobs[1]
Department of Pathobiology[1], University of Guelph, Abbott Laboratories, USA[2], Animal Health Laboratory[3] and Department of Pathology, Microbiology and Immunology, University of California, Davis[4]

Canine Cutaneous Histiocytoma (CCH), a benign neoplasm of Langerhans cells, is one of few naturally-occurring tumors that display spontaneous immune-mediated regression. This unique model for effective cell-mediated anti-tumor immunity undergoes regression via lymphocytic inflammation dominated by CD8+ cytotoxic lymphocytes (CTL). Perforin is a key component of CTL-mediated killing. This study aims to evaluate the role of perforin in CCH regression. Anti-canine perforin antibody was generated by immunization of rabbits using a novel perforin peptide. Antibody specificity was assessed by immunoblot analysis. Immunohistochemistry (using this antiserum) was performed on lymphoid-rich tissues from healthy dogs and CCH biopsies, along with differentiating lymphoid markers (CD3& CD79a). Immunoblotting identified a band at ~65kD, corresponding to the published weights

of human and murine perforin, and to the predicted weight of canine perforin. Perforin expression was detected in CD3+ but not CD79a+ lymphocytes. We showed that CCH regression is characterized by a consistent presence of perforin-expressing T-cells normally absent in lymph nodes, spleen and intestinal lymphoid aggregates. Also, although CCH lymphocytic infiltrates are known to be dominated by CTL, only a small proportion express perforin, a probable reflection of heterogeneous activation states. These findings suggest a role for perforin expression in the course of CCH regression.

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7) Guelph Companion Animal Epidemiologic Cancer Registry (CAnCER).
Kathleen Yates and Olaf Berke, Department of Population Medicine, University of Guelph

Although cancer is the most common fatal disease among dogs, accurate population-based incidence rates of specified cancers are not available. Veterinary cancer registries are few in number and experimental projects. Results from those projects show a marked variation in cancer incidence. The Guelph CAnCER is designed as a population-based registry and will register all newly occurring neoplasia in dogs and cats that reside within the City of Guelph. Cancer case information is submitted to the registry via Internet by participating veterinary clinics upon owner consent. Information from the Teaching Hospital at OVC is retrieved from its medical record system. The size of the dog population is estimated based on the number of licensed dogs in Guelph in combination with a capture-recapture method. The cat population is estimated based on dog-to-cat ratio statistics. The registry project is approved by the University of Guelph Research Ethics Board. It started January 2010 with support from 14 of 15 recognized veterinary practices in the Guelph area. Guelph CAnCER will further our understanding of cancer incidence in pets and success of treatments. In addition, the registry may provide a basis for human cancer research due to the comparative aspects of certain canine and feline cancers.

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8) Characterizing food-food synergism to optimize anti-cancer activity in MCF-7 breast cancer cells.
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Combinations of different foods may produce additive, synergistic or antagonistic interactions that may modify the pharmacological effects such as the anticancer properties of foods or diets. In order to investigate these interactions and identify potential synergetic combinations, thirteen different foods from three categories, including culinary fruits (raspberries, blackberries, apples and grapes), culinary vegetables (broccoli, tomatoes, mushrooms, purple cauliflowers and onions) and legumes (soy beans, adzuki beans, red kidney beans and black beans) were evaluated to determine their inhibitory activity in breast cancer cells MCF-7. Grape, onion and adzuki bean showed maximal inhibition of the growth of MCF-7 in the fruits, vegetable and legumes groups, respectively. When these three foods were combined in pairs, unique food-food interactions were observed, not seen when

individual extracts were used. The combination of onion and grapes resulted in a synergistic anti-proliferative effect against MCF-7 compared to that of onion and grapes treatment alone. In contrast the combination of grape and adzuki bean resulted in antagonism. Further investigation is required to determine the mechanism by which these unexpected interactions occur and how to predict and enhance the therapeutic benefits of foods and food components by using strategic food combinations.

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9) Prospective evaluation of clinically relevant type B hyperlactatemia in dogs with cancer. *M Touret[1], SR Boysen[2], ME Nadeau[3]; [1]Clinical Studies, University of Guelph; [2]Faculty of Veterinary Medicine, University of Calgary; [3]Department of Oncology, Centre Hospitalier Universitaire Vétérinaire, Université de Montréal, Saint-Hyacinthe, Québec*

Background: Cancer is considered a cause of type B hyperlactatemia in dogs but studies are lacking. It is well accepted that cancer cells have a higher lactate production due to increased aerobic glycolysis ("Warburg effect"). The mechanisms are not well elucidated but it has been suggested that neoplasia may cause type B hyperlactatemia via this process.
Objectives: To determine if canine malignant tumors could be associated with a clinically relevant type B hyperlactatemia (> 2.5 mmol/L).
Methods: Thirty-seven dogs with malignant tumors were included: 22 with hematopoietic and 15 with solid tumors. Histology was used to confirm the diagnosis (cytology was considered appropriate for lymphoma). Confounding factors associated with hyperlactatemia were excluded. Lactate measurements were obtained from a free flow jugular whole blood sample and immediately analyzed using the LactatePro®.
Results: All dogs had lactate values less than 2.5 mmol/L. The mean blood lactate concentration was 1.09 mmol/L (0.95mmol/L, 1.19mmol/L and 1.15mmol/L respectively for solid, hematopoietic tumors and lymphoma).
Conclusions: Malignant tumors were not considered a cause of clinically relevant type B hyperlactatemia. Therefore, cancer related type B hyperlactatemia in dogs with cancer is uncommon and its diagnosis should prompt careful investigation for causes other than cancer.

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10) Cannabidiol, a nonpsychoactive component of cannabis, attenuates vomiting and nausea by activating 5-HT_{1A} somatodendritic autoreceptors in the dorsal raphe nucleus (DRN).
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Cannabidiol (CBD), a non-psychoactive component of cannabis, was effective in suppressing nicotine-, lithium chloride (LiCl)- and cisplatin (20 mg/kg, but not 40 mg/kg)-induced vomiting in the Suncus murinus (house musk shrew) and LiCl-induced conditioned gaping (a nausea-like behaviour) in rats. The anti-emetic and anti-nausea effects of CBD were

suppressed by systemic pre-treatment with the 5-hydroxytryptamine 1A (5-HT_{1A}) receptor antagonist WAY100135. In addition, the more selective 5-HT_{1A} receptor antagonist, WAY100635, administered systemically or intracranially into the dorsal raphe nucleus (DRN), a site of somatodendritic 5-HT_{1A} autoreceptors, interfered with the CBD-induced suppression of LiCl-induced conditioned gaping in rats. These results suggest that CBD produces its anti-emetic/anti-nausea effects by agonism of somatodentritic autoreceptors located in the DRN, potentially by reducing the release of forebrain 5-HT.

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11) TIMP3 Deficiency Promotes Invasive Prostatic Carcinoma

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Deregulated expression and activity of proteases is implicated in cancer progression. An important negative regulator of protease activity is TIMP3 (tissue inhibitor of metalloproteinase 3). TIMP3 expression is lacking in many cancers including advanced prostate cancers, and this may facilitate invasion and metastasis by allowing unrestrained protease activity. To investigate the role of TIMP3 in prostate cancer progression, we crossed whole-body TIMP3 deficient mice (timp3-/-) with a mouse model of prostate cancer (mice with prostate-specific deletion of the tumour suppressor pten; pten-/-). Assessment of prostate cancer development in timp3 wild type versus timp3-/- mice at 16 weeks of age showed that timp3-deficiency promoted tumor growth and cell proliferation, increased microvascular density, and increased invasion. Tumor invasion in TIMP3-deficient mice was associated with increased matrix metalloproteinase (MMP)-2 activation and increased MMP-9 expression. Further, there is markedly increased inflammatory cell influx into the TIMP3-deficient prostate tumors along with increased expression of monocyte chemoattractant protein 1 (MCP-1, also called CCL2) and Interleukin-1β (IL-1β), cytokines implicated in both inflammation and cancer. Similar results were also observed in timp3+/- mice indicating haploinsufficiency for timp3 protein in prostate cancer progression. This study provides important insights into the roles of proteases and inflammation in cancer progression, and shows the potential of restoring/augmenting TIMP3 activity as a novel therapeutic approach for inflammation-associated neoplasia.

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12) Ischemia dysregulates DNA methyltransferases and *p16INK4a* methylation in human colorectal cancer cells.

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Epigenetic modifications are involved in the initiation and progression of cancer. Expression and activity of DNA methyltransferases (DNMTs) are strictly controlled in normal cells, however, regulation of these enzymes is lost in cancer cells due to unknown reasons. Therapies which target DNMTs show promising results in the treatment of hematologic cancers, but lack the same effectiveness in solid tumors. Solid tumors exhibit areas of

hypoxia and hypoglycaemia due to their irregular and dysfunctional vasculature. Here we show that hypoxia reduces global DNA methylation in different human cancer cell lines and xenografts. Colorectal carcinoma cells (CRC) (HCT116 and 379.2, $p53^{+/+}$ and $p53^{-/-}$, respectively) were further subjected to ischemia (hypoxia and hypoglycaemia) in vitro, and levels of DNMTs were assessed. This produced significant decreases in mRNA for DNMT1, DNMT3a and DNMT3b, and similar reductions in DNMT1 and DNMT3a protein levels were detected by western blotting. In addition, total activity levels of DNMTs (as measured by an ELISA-based DNMT activity assay) were reduced in cells exposed to hypoxic and hypoglycaemic conditions. Immunofluorescence of HCT116 tumor xenografts demonstrated an inverse relationship between ischemia (as revealed by carbonic anhydrase IX staining) and DNMT1 protein. To examine the functional consequences of ischemia-repressed DNMT expression and activity, bisulfite sequencing of the *p16INK4a* promoter region was performed and showed a decrease in 5-methylcytosine following in vitro exposure to ischemia. This study provides evidence for the down-regulation of DNMTs and modulation of methylation patterns by hypoxia and hypoglycaemia in human CRC cells, both in vitro and in vivo.

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13) The role of type-I insulin-like growth factor receptor in lung cancer

S Elizabeth Franks, Megan D Siwicky, Roger A Moorehead, Biomedical Sciences, University of Guelph

The type I insulin-like growth factor receptor (IGF-IR) has been implicated in many types of cancer and is frequently expressed at high levels in lung cancer. This receptor is involved in cell proliferation, apoptosis, migration and differentiation and is emerging as a potential target for molecular therapies. In our lab, doxycycline-inducible transgenic mice have been generated in which over-expression of IGF-IR in type II alveolar or Clara cells leads to the development of lung tumours, indicating that this receptor has an important function in the initiation of lung tumorigenesis. To further investigate IGF-IR in lung cancer, murine lung cancer cells (RLEJenvC1) and normal rodent lung epithelia (RLE) cells were used. Western blotting revealed increased levels of IGF-IR and phosphorylated downstream molecules Akt and ERK1/2 in RLEJenvC1 cells compared to the untransformed RLE cells, confirming the importance of IGF-IR signalling in these cells. Down-regulation of IGF-IR using RNAi also decreased the levels phosphorylated Akt. Furthermore, treatment of RLEJenvC1 cells with a small molecule inhibitor of IGF-IR, BMS-754807, led to decreased cell survival in a dose dependent manner. Future studies will determine the influence of IGF-IR on proliferation, apoptosis and migration of RLEJenvC1 cells *in vitro* and *in vivo*.

14) The effect of lipid peroxidation on JNK activation and GSTA1-JNK complex dissociation in the human colon adenocarcinoma Caco-2 cells

Humaira Adnan, Monica Antenos, Gordon Kirby, Department of Biomedical Sciences, University of Guelph.

Glutathione-S-transferase alpha (GSTA1) plays an important cytoprotective role in detoxifying reactive electrophiles and products of lipid peroxidation. GSTs are inhibitors of stress activated kinases, such as c-jun N-terminal kinase (JNK). JNK activation is inhibited in non-stressed cells by forming complexes with GSTA1. Altered levels of GSTA1 protein may be an important factor in modulating the cellular transition between proliferation, differentiation and apoptosis. Stress signals that cause GSTA1 dissociation from JNK have not been identified. We hypothesized that the products of lipid-peroxidation may activate JNK and result in GSTA1-JNK dissociation. To test this, pre- and post-confluent Caco-2 cells were treated with increasing concentrations of the pro-oxidant, menadione. We demonstrate that with increasing concentrations of menadione, a concomitant increase in p-JNK was observed. Time course studies determined that maximal p-JNK activation was observed 3 h after menadione treatment in pre-confluent cells only. We next assessed the levels of GSTA1 protein in the GSTA1-JNK complex in pre-confluent cells treated with menadione. Less GSTA1 protein was observed in complex with JNK after a 30 minute treatment with menadione, suggesting the dissociation of GSTA1 and JNK with lipid peroxidation. The data suggest that lipid peroxidation acts as a stimulus for GSTA1-JNK dissociation and JNK activation.

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15) Mapping drug interactions inside the substrate-binding pocket of P-glycoprotein

David Ward, Frances J. Sharom, Department of Molecular and Cellular Biology, University of Guelph

P-Glycoprotein (Pgp, ABCB1 in humans), is a 170 kDa polyspecific multidrug transporter and has been implicated in the development of multidrug resistance (MDR) in many human cancers, where it transports a number of chemotherapeutic drugs out of tumour cells, limiting their cytotoxicity. The drug-binding pocket of Pgp appears to be large and flexible, with several overlapping sub-sites, and both the X-ray crystal structure and biochemical studies indicate that two drug molecules can bind simultaneously. The R-site drug azido-tetramethylrosamine (AzTMR) was crosslinked to Pgp with a drug:protein stoichiometry of 1, and the binding affinity of other substrates to the Pgp-drug adduct was estimated by quenching of the intrinsic Trp fluorescence. Results revealed that a second drug molecule may bind with lower affinity when compared to native Pgp, suggesting partial overlap of the sub-site where the drug binds with the site of TMR linkage. In contrast, some drugs, and large (>750 Da) drug dimers joined by an aliphatic linker, bind to Pgp-AzTMR with higher affinity, providing evidence of binding cooperativity. Understanding the biochemical basis of drug binding to Pgp will facilitate the design of selective Pgp inhibitors to reverse MDR in clinical oncology.

16) Development of polymeric nanoparticles for targeted cancer therapy

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There has been progressively heightened interest in the development of targeted nanoparticles (NPs) for differential delivery and controlled release of drugs. Despite nearly three decades of research, approaches to reproducibly formulate targeted NPs with the optimal biophysicochemical properties that result in a desirable biodistribution and drug release profiles have remained elusive. Here we report a potentially scalable strategy for narrowly changing the biophysicochemical properties of NPs in a reproducible manner, thereby enabling systematic screening of optimally formulated drug-encapsulated NPs. NPs were formulated by the self-assembly of an amphiphilic triblock copolymer comprised of end-to-end linkage of poly(lactic co-glycolic acid) (PLGA), polyethyleneglycol (PEG) and the A10 aptamer (Apt) which binds to the extracellular domain of the prostate specific membrane antigen (PSMA) on the surface of prostate cancer (PCa) cell, enabling respectively, controlled drug release, immune evasion and cell-specific targeting. Fine-tuning of NP size and drug release kinetics was further accomplished by controlling the copolymer composition. Using distinct ratios of PLGA-b-PEG-b-Apt triblock copolymer with PLGA-b-PEG diblock copolymer lacking the A10 Apt, we developed NPs with varying Apt surface density and identified the narrow range of Apt density for maximum PCa cell uptake in vitro and in vivo. The use of this triblock copolymer approach demonstrated that the NP biophysicochemical properties may be systematically fine tuned within a narrow range requiring no additional chemistry after their self assembly. The nanoparticle size and drug release properties can be further optimized by controlling the rate of nanoparticle formation. A typical synthesis of triblock copolymer nanoparticles by nanoprecipitation invovles slow and uncontrolled mixing. We demonstrate that rapid and tunable microfluidic mixing can be used to synthesize drug-encapsulated nanoparticles with defined size, lower polydispersity, and higher drug loading. These results may contribute to further development of targeted NPs as highly selective and effective therapeutic modalities.

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17) Examination of sickness behaviour in dogs with lymphoma being treated with CHOP chemotherapy – a pilot study.

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Introduction: Chemotherapy is a common treatment for dogs with lymphoma; however, chemotherapy may result in alterations in behaviour. Little research has focused on the behavioural changes. Hence, the purpose of this project was to investigate sickness behaviour in dogs with lymphoma treated with chemotherapy.

Methods: Dogs diagnosed with lymphoma and treated with CHOP chemotherapy were enrolled in the study. Questionnaires, physical exams, and Actical activity monitors were utilized to acquire information regarding activity level, anxiety, pain, appetite, and side effects of the dogs.

Results: Sixteen dogs were enrolled, with data available from 8 dogs that completed the study. Owners reported (on a 5 point scale) a 2.5 point increase in welfare and a 2 point increase in energy between the beginning and end of the chemotherapy induction phase. These dogs also exhibited an improvement in overall clinical condition improving from a restricted condition to a normal condition. Actical activity data revealed no difference.
Conclusions: These dogs experienced an increased quality of life during the induction phase of chemotherapy. Due to the apparent connection between clinical signs and biologic mechanisms, description of cancer-related signs and correlation of these signs with clinical laboratory data is an important area for future research studies.

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18) The Role of MicroRNAs in Mammary Tumorigenesis
Erinne Barnett, Roger Moorehead, Biomedical Sciences, University of Guelph

MicroRNAs (miRNAs) are small non-coding regulatory RNA molecules that act post-transcriptionally to regulate the expression of most cellular genes, and their expression has been implicated in the development of breast cancer. Using a transgenic mouse model for mammary tumorigenesis, a miRNA array was performed to study the expression level of various miRNAs, which were found to be differentially upregulated in mammary tumours compared to wild-type mammary tissue. Using a cell line (RM11A) generated from MTB-IGFIR transgenic mice, the presence of several miRNAs were confirmed with real-time PCR. Additionally, we have shown that cell transfection with synthetic miRNA precursors and inhibitors, that enable specific miRNA overexpression, and inhibition, respectively, can regulate the expression levels of miR-210 and miR-378. Proliferation, as assessed by Ki67 immunofluorescence, revealed that overexpression of either miR-210 or miR-378 decreased proliferation. Future experiments will determine the effects of miR-210, miR-378 and other miRNAs on migration using a Boyden chamber assay, and apoptosis using activated caspase-3 immunofluorescence. Discovering the differential expression of miRNAs and their effects on proliferation, migration, and apoptosis in the RM11A cells will enhance our understanding of the function of miRNAs in human breast cancer.

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19) Canine Subcutaneous Mast Cell Tumors: Proliferation Markers and c-KIT Evaluation as Prognostic Indices
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Various molecular assays are widely used to prognosticate canine cutaneous mast cell tumors (MCT). There is limited data as to whether these prognostic assays can be routinely applied to MCT that arise in the subcutis. The aims of this study were to evaluate the utility of KIT immunohistochemical pattern, *c-KIT* mutational status (presence of internal tandem duplications in exon 11) and proliferation markers, including mitotic index (MI), Ki67, and argyrophilic nucleolar organizing regions (AgNOR), as independent prognostic markers for

local recurrence and/or metastasis in canine subcutaneous MCT. A case-control design was used to analyze 60 subcutaneous MCT from 60 dogs, consisting of 24 dogs with subsequent local recurrence and 12 dogs with metastasis compared to age, breed and sex-matched dogs that did not experience these events. Proliferation indices (MI, Ki67, AgNOR) and KIT pattern were significantly associated with both local recurrence and metastasis, demonstrating their prognostic value for subcutaneous MCT. No internal tandem duplication mutations were detected in exon 11 of *c-KIT* in any tumors. Since *c-KIT* mutations have only been demonstrated in 15% of cutaneous MCT and primarily in tumors of higher grade, the number of subcutaneous MCT analyzed in this study may be insufficient to draw conclusions on the role *c-KIT* mutations in these tumors.

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20) Molecular cytogenetic investigation of mouse epithelial ovarian tumors - first results

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Ovarian cancer is the most common malignancy of the female reproductive tract and is the most lethal gynecological cancer. This poster summarizes the preliminary results of the molecular cytogenetic comparison of a Murine Ovarian Surface Endothelial Cell line (MOSEC, ID8) and the cell line (28-2) established from the secondary lesions of the primary ovarian tumors developed from ID8 cells injected under the bursa. Conventional cytogenetic screening showed gross numerical aberrations (2n=72-75) confirmed by flow cytometric measurement of total nuclear DNA content. To characterize the expected complex cytogenetic alterations we performed spectral karyotyping (SKY). This most precise multicolor FISH (fluorescent in situ hybridization) technique makes possible the recognition of all chromosomes by painting probes identified by the spectrum of each image pixel. The results showed complex chromosomal rearrangements, severe aneuploidy, translocations, deletions, sporadic centromere amplification in both cell lines. The comparison of the SKY karyotypes between the two cell lines confirms the ID8 origin of most of the numerical abnormalities and translocations, but points to several aberrations generated only in the 28-2 cell line, maybe responsible for its more aggressive tumorigenesis and other previously observed properties and reflect the interaction of tumorigenic epithelial cells with the ovarian stroma microenvironment.