INSTITUTE FOR COMPARATIVE
CANCER INVESTIGATION

12th Annual ICCI

Cancer Research Symposium

Tuesday May 28, 2019

OVC LLC 9:00-5:00

Introductory Remarks

Welcome to the 12th annual Guelph ICCI Cancer Symposium! This meeting is an opportunity to bring together cancer researchers from across campus and regional collaborators. Topics range from basic science through to clinical application. We are very grateful to the amazing group of speakers and poster presenters who will be sharing their findings with us today. Dr. David Vail is the 2019 Arthur Willis Distinguished speaker and will be giving the keynote address at 3:00.

In the past 12 years we have seen relationships and collaborations develop that were made possible by these interactions and we hope that this year's meeting will spark new collaborations and ideas.

This symposium is made possible by funding from the Arthur Willis Visiting Professorship in Canine Oncology and support from the OVC Dean's office.

Drs Geoff Wood and Michelle Oblak
Pathobiology and Clinical Studies, University of Guelph
ICCI Assistant Co-Directors

Administrative Support and Research Funding:

Thanks to Dr. Kaya Skowronski from the ICCI tumor bank for huge help organizing this symposium along with support from Barb Gaudette and Daphne Summers from the OVC Office of the Dean, Hospitality Services for help with set up and refreshments, and Marina Kashevska-Gozdek and Melanie Knapp for assistance with the program and organization throughout the day.

The research projects presented here and the trainees performing these studies were collectively supported by grants, scholarships and contracts from: CIHR; NSERC; Terry Fox Research Institute; OGS; Cancer Research Society; OVC Pet Trust Fund; Smiling Blue Skies Cancer Fund; OVC Graduate Scholarship; Art Rouse Cancer Biology Graduate Stipend; BioCanRx; Vanier Canada Graduate Scholarship; Brock Doctoral Scholarship; QEII-GSST, Government of Ontario, Stem Cell Network, and the CanaQuest Medical Corporation Cancer Research Program.

ICCI 12th Annual Cancer Research Symposium, Tuesday May 28th, 2019

Morning Session: Room 1714, OVC LLC

9:00-9:05 Welcome and Introductory Remarks

9:05-9:35 Guest Speaker
Utilizing vascular normalization to increase the uptake, efficacy, and impact of cancer therapies
Dr. Jim Petrik; Department of Biomedical Sciences, University of Guelph

9:35- 10:20 Short talks from abstracts

1. *Viral sensitizer-mediated enhancement of oncolytic NDV leads to rapid clearance of primary tumours in a mouse model of melanoma*
   Thomas McAusland; Department of Pathobiology, University of Guelph
2. *Combining Decitabine with Oncolytic Virotherapy Preferentially Kills Acute Myeloid Leukemia Cells Via Lethal Oxidative Stress*
   Elaine Klafuric; Department of Pathobiology, University of Guelph
3. *Investigating the potential to target colorectal cancer-linked Fusobacterium nucleatum with Bdellovibrio and like organisms*
   Avery Robinson; Department of Molecular and Cellular Biology, University of Guelph

10:20-10:45 ***Coffee Break and Poster Viewing*** **Room 1707 B & C, OVC LLC**

10:45-11:30 Short talks from abstracts

1. *Analysis of SNARE Regulation During Tumor Cell Invasion*
   Megan Brasher; Department of Molecular and Cellular Biology, University of Guelph
2. *Characterization of extracellular vesicles obtained from cultured explants of canine osteosarcoma and normal bone*
   Dr. Alicia Viloria-Petit; Department of Biomedical Sciences, University of Guelph
3. *Lymphoma and Symmetric Dimethylarginine Concentraion in Dogs*
   Dr. Anthony Abrams-Ogg, Department of Clinical Studies, University of Guelph

11:30-12:20 Regional Keynote Speaker
Regulation of the Hippo pathway by AMPK family kinases in cancer
Dr. Liliana Attisano; Department of Biochemistry, University of Toronto

12:20- 1:30 ***Poster Session and Lunch*** **Room 1707 B & C, OVC LLC**

Afternoon Session: Room 1714, OVC LLC

1:30-1:55 Guest Speaker

A comparative oncology approach to investigate the Eph receptor tyrosine kinases as novel targets for cancer therapy in companion animals and humans

Dr. Behzad Toosi; Department of Small Animal Clinical Sciences, University of Saskatchewan

1:55- 2:40 Short talks from abstracts

1. *Targeting Mitochondrial Metabolism with Avocatin-B Induces Selective Acute Myeloid Leukemia Death*
   Matthew Tcheng; Department of Food Sciences, University of Guelph
2. *Diet Type and Supplement Use in Healthy Dogs and Dogs with Cancer*
   Adriana Bianco; Department of Clinical Studies, University of Guelph
3. *Effects of cannabinoids and marine oil derivatives on cell migration and tumour formation in colorectal cancer models*
   Dr. Jonathan Blay; School of Pharmacy, University of Waterloo, ON

2:40-3:00 ***Coffee break*** **Room 1707 B & C, OVC LLC**

3:00 - 4:00 Keynote Speaker**,** introduced by Dr. Paul Woods

Comparative Cancer Immunotherapy Trials: A One Medicine Approach

Dr. David Vail, DVM, Diplomate ACVIM (Oncology), Professor and Barbara A. Suran Chair in Comparative Oncology, University of Wisconsin-Madison, USA

4:00-5:00 ***Closing Remarks and Reception*** **Room 1707 B & C, OVC LLC**

Snacks, poster award ceremony and last chance for poster viewing

KEYNOTE PRESENTATION

3:00 OVC LLC Room 1714

Dr. David M. Vail, DVM, Diplomate ACVIM (Oncology)

Professor and Barbara A. Suran Chair in Comparative Oncology University of Wisconsin-Madison

Comparative Cancer Immunotherapy Trials: A One Medicine Approach

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Although immunotherapy is becoming one of the cornerstones of modern cancer therapy, the majority of patients still fail to experience durable responses. This is further compounded by our inability to predict or assess response owing to unusual response patterns unique to immunotherapy. Recently, the scientific community has begun to explore the possibility that the inclusion of companion species (companion dogs) in clinical trials of novel immunotherapeutic agents, combinations of agents, and the assessment of response to immunotherapy may hold promise. This stems from the fact that companion dogs have intact immune systems and heterogenous tumour/tumour microenvironments which may better recapitulate the human condition. Several examples of current cancer immunotherapy trials in pet dogs will be presented to illustrate potential advantages and pitfalls of the comparative approach.

Dr. Vail received his DVM from the University of Saskatchewan in 1984 and subsequently completed an internship in small animal medicine and surgery at Colorado State University prior to practicing in his native Edmonton for two years. He followed up with a residency in Medical Oncology at the Animal Cancer Center at Colorado State University, completed in 1990. He is currently Professor and Barbara A. Suran Chair in Comparative Oncology at the University of Wisconsin-Madison and a member of the UW Carbone Comprehensive Cancer Center. Dr. Vail has published over 150 peer-reviewed scientific manuscripts and 50 book chapters in the field of veterinary and comparative oncology. David is co-editor of the textbook Small Animal Clinical Oncology. He has served in the past as President of the Veterinary Cancer Society, the Canine Comparative Oncology and Genomics Consortium (CCOGC), Chairman of the Scientific Advisory Boards for both the Morris Animal Foundation and the American College of Veterinary Internal Medicine Foundation, and North American journal editor for Veterinary and Comparative Oncology. He is a founding member of the Comparative Oncology Trials consortium. Dr. Vail has been honored as the recipient of both the Mark L. Morris Sr. Distinguished Research Award and the Pfizer Award for Veterinary Research Excellence.

Past ICCI Symposium Arthur Willis Distinguished Speakers

2018	Daniel Gustafson	2013	David Argyle
2017	William Eward	2012	Timothy Fan
2016	Jaime Modiano	2011	Cheryl London
2015	Nicola Mason	2010	Matthew Breen
2014	Deborah Knapp	2009	Barbara Kitchell

GUEST SPEAKER:

9:05-9:35

Utilizing vascular normalization to increase the uptake, efficacy, and impact of cancer therapies

Dr. Jim Petrik; Department of Biomedical Sciences, University of Guelph

Tumors often initiate an aggressive program of angiogenesis in order to accommodate the oxygen and metabolic needs of rapidly-growing tissue. As a result of the strong pro-angiogenic stimulus, blood vessels form very rapidly and often are disorganized, immature, and dysfunctional. This dysfunctional vasculature reduces perfusion and leads to areas of hypoxia and elevated interstitial fluid pressure. All of these characteristics impede drug delivery to the tumor, significantly reducing the efficacy of anti-cancer therapy and necessitating the administration of high levels of compound in order to have some uptake in to the tumor. We have developed an approach to target this immature, dysfunctional tumor vasculature through the use of the type I repeat region (3TSR) of thrombospondin-1 (TSP-1). TSP-1 is a large matricellular glycoprotein with potent endogenous anti-angiogenic activity. We have shown previously that 3TSR specifically targets the immature tumor vasculature in primary and metastatic ovarian tumors. 3TSR also causes apoptotic tumor cell death. As a result of this bimodal function, 3TSR stimulates regression of advanced stage ovarian cancer and induces tumor shrinkage, enhances vascular perfusion, and reduces tumor hypoxia. Our previous data with 3TSR has shown that vascular normalization can enhance the uptake and efficacy of a number of different therapeutic compounds and immune cells. However, the small size of 3TSR results in rapid clearance from circulation and a serum half-life of approximately 14hrs. Here, we discuss the development of a Fc fusion protein (Fc3TSR), which links two 3TSR molecules with a human IgG protein. The half-life of Fc3TSR is approximately 8 days, which makes the compound more attractive clinically. In addition to increased time in circulation, Fc3TSR also has better in vitro efficacy, potentially due to enhancing clustering of its receptor CD36. This presentation will focus on the use of vascular normalization to improve anti-cancer therapy and the development of Fc3TSR which we hope to take to the clinic, initially for the treatment of advanced stage ovarian cancer.

REGIONAL KEYNOTE SPEAKER:

11:30-12:20

Inhibition of the Hippo pathway by AMPK-family kinases

Dr. Liliana Attisano; Donnelly Centre and Department of Biochemistry, 160 College Street, University of Toronto

The Hippo signalling pathway is a key regulator of tissue growth and organogenesis. The pathway is comprised of a core MST/LATS kinase cassette that phosphorylates and promotes cytoplasmic localization of the transcriptional regulators, TAZ and YAP. Inactivation of the Hippo pathway is a common feature in numerous cancers, yet mutations in pathway components are relatively rare. To uncover novel Hippo pathway regulators, we conducted multidimensional high throughput screens. These efforts uncovered two AMPK family kinases, MARK4 and NUAK2 as negative regulators of the Hippo pathway. MARK kinases, including MARK3 and MARK4, phosphorylate

both SAV1 and MST1/2 and inhibit MST1/2-dependent activation of LATS. Moreover, we showed that DLG5, acts as a scaffold to promote MARK-mediated phosphorylation of MST. In contrast to MARKs, the AMPK family member, NUAK2 interacts with and phosphorylates LATS. Interestingly, NUAK2 is induced by YAP/TAZ in cooperation with AP-1 and this is required for robust YAP/TAZ signalling. Inhibition or loss of NUAK2 reduces the growth of cultured cancer cells and mammary tumors in mice. In human patient samples, *NUAK2* expression is elevated in aggressive, high grade bladder cancer and strongly correlates with a YAP/TAZ gene signature. Thus, we identified a positive feed forward loop in the Hippo pathway that establishes a key role for NUAK2 in enforcing the tumour promoting activities of YAP/TAZ. Developing inhibitors for therapeutic applications and understanding how these AMPK family members act to regulate the Hippo pathway in distinct physiological contexts and their impact in human disease are outstanding questions.

GUEST SPEAKER:

1:30-1:55

A comparative oncology approach to investigate the Eph receptor tyrosine kinases as novel targets for cancer therapy in companion animals and humans

Dr. Behzad Toosi**;** Department of Small Animal Clinical Sciences, University of Saskatchewan

Naturally occurring tumors that develop in companion animals are unfortunate for both the animal and the pet owner but represent an overlooked opportunity to facilitate the search and discovery of new diagnostics and therapies for human malignancies. Testing of new therapies in animal models that better represent human disease, such as naturally occurring tumors in pet dogs, has the capacity to reduce the time for clinical development of new pharmaceutical agents for human cancer therapy. At the same time, there exists an opportunity to bring the novel and advanced diagnostics and therapeutics from human drug discovery and pharmaceutical research to veterinary medicine. Our team is focused on identification of new cancer biomarkers of common canine and human neoplasms and on the validation of spontaneous canine models of human malignancies based on the role of receptor tyrosine kinases.

Protein kinases have been commonly identified as oncogenes because various cancers show a high frequency of activating mutations in the genes encoding these proteins. Accordingly, receptor tyrosine kinases represent promising targets for the development of new cancer therapies that include monoclonal antibodies and small molecule inhibitors. The Eph family of receptor tyrosine kinases with 14 members are often overexpressed in several human malignancies and their function has been associated with tumor growth, invasiveness and metastasis. Our laboratory investigates multiple canine spontaneous models of human cancers in terms of Eph receptor expression, signaling mechanisms and function, and the regulation of response to treatment to identify new diagnostics and therapies that will benefit both companion animals and humans.

SHORT TALKS FROM SUBMITTED ABSTRACTS

9:35-10:20 Morning Session

Viral sensitizer-mediated enhancement of oncolytic NDV leads to rapid clearance of primary tumours in a mouse model of melanoma

Thomas M. McAusland, Jacob P. van Vloten, Lisa A. Santry, Joelle C. Ingrao, Matthew M. Guilleman, Amira D. Rghei, Leo Susta, Khalil Karimi, and Byram W. Bridle and Sarah K. Wootton
Department of Pathobiology, University of Guelph, Guelph, Ontario

The avian paramyxovirus, Newcastle disease virus (NDV), is a potent oncolytic virus that has been shown to be safe and effective in a variety of preclinical cancer models as well as in human clinical trials. NDV preferentially replicates in and lyses tumour cells while sparing normal cells. In addition, NDV possesses strong immunostimulatory properties that can overcome cancer-induced immunosuppression and generate effective anti-tumour immune responses. The oncolytic efficacy of NDV is negatively impacted by tumours that retain intact antiviral signalling and sensing capabilities. The objective of this research was to evaluate the use of viral sensitizer-mediated combination therapy to enhance the anti-neoplastic efficacy of NDV. Here, we demonstrate that treatment with a combination of NDV and viral sensitizer causes rapid regression and clearance of B16-F10 tumours following intratumoral injection. In addition, we determined that the anti-tumour efficacy of this combination therapy is reliant upon the actions of natural killer (NK) cells as antibody depletion of NK cells abrogated therapeutic efficacy in this model. This viral sensitizer does not act to increase virus replication, but rather acts as an immune-potentiator to activate cells of the innate immune system to rapidly clear primary tumours. Although this treatment strategy is reliant on the activity of NK cells, it is suspected that type I IFN also plays an important significant role and we are currently investigating this mechanism. Taken together, these results suggest that combining NDV with a viral sensitizer alerts the innate immune system to the presence of primary tumours, and this in turn facilitates rapid tumor clearance.

Funding: OVC Scholarship

Combining Decitabine with Oncolytic Virotherapy Preferentially Kills Acute Myeloid Leukemia Cells Via Lethal Oxidative Stress

Elaine M. Klafuric[1,*], Megan R. Strachan-Whaley[1,*], Lisa Santry[1], Amanda W.K. AuYeung[1], Jacob P. van Vloten[1], Robert C. Mould[1], Thomas McAusland[1], Khalil Karimi[1], Anthony J. Mutsaers[2,3], Sarah K. Wootton[1] and Byram W. Bridle[1]

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[*]contributed equally

Acute myeloid leukemias (AML) are aggressive hematological cancers for which the standard of care has limited efficacy, with high rates of relapse. The DNA methyltransferase inhibitor decitabine is an epigenetic modifier in clinical trials to treat leukemias, albeit with limited efficacy. Oncolytic viruses (OVs) preferentially replicate in and kill cancer cells but perform poorly against leukemias that are spread throughout normal tissues that can quench viral infections. However, we discovered that combining decitabine with OVs induced durable remissions and resistance to relapse in mouse models of acute T- and B-lymphocytic leukemias. Therefore, we hypothesized that treatment with decitabine would sensitize AML cells to killing by oncolytic Newcastle disease virus (NDV). *In vitro* resazurin dye-based assays supported the hypothesis. Further, most mice challenged with C1498 AML cells and treated with decitabine seven and eight days later, followed by NDV eleven days post-challenge, achieved durable remissions and resisted a homologous re-challenge. Co-administration of the pan-reactive oxygen species (ROS) scavenger N-acetyl-L-cysteine abrogated efficacy. This implicated induction of lethal oxidative stress as a mechanism of action. Flow cytometric detection of ROS suggested that decitabine and NDV caused oxidative stress in leukemia cells, with the combination therapy having an additive effect. More specific reagents, such as dihydroethidium and mitoSOX, will be used to quantify cytoplasmic versus mitochondrial ROS, like superoxide. Targeted ROS inhibitors will be employed to confirm which subtypes of ROS are involved. In conclusion, treatment with clinically-approved decitabine followed by NDV appears to be effective at preferentially killing AML cells via oxidative stress.

Funding: Operating funds were jointly provided by the Canadian Cancer Society Research Institute and Canadian Institutes of Health Research-Institute of Cancer Research via an Innovation Grant to BWB. Stipend funding: Ontario Veterinary College (OVC) Graduate Scholarship (EMK); Canadian Institutes of Health Research Graduate Scholarship (Masters Award) and OVC Pet Trust Scholarship (AWKAY); Ontario Graduate Scholarship and OVC Graduate Scholarship (JPvV); OVC Pet Trust Scholarship (RCM); OVC Graduate Scholarship (TM).

Investigating the potential to target colorectal cancer-linked *Fusobacterium nucleatum* with *Bdellovibrio* and like organisms

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The etiology of colorectal cancer (CRC) is highly complex: most cases of CRC are sporadic, diagnosed in patients with no family history or genetic predisposition. One contributing factor to sporadic CRC development is the human microbiome. *Fusobacterium nucleatum* is a Gram-negative bacterium typically found as an oral commensal species. Yet, *F. nucleatum* has been isolated at high abundance from cancerous colon mucosae and thus linked to CRC. *Bdellovibrio* and like organisms (BALOs) are relatively small bacteria that hunt and prey upon other Gram-negative bacterial cells. BALOs present an attractive potential application in human disease as [1] BALOs do not damage human cells and [2] BALOs are proficient at invading and disintegrating bacterial biofilms, penetrating through sessile bacterial cells far more efficiently than antibiotics or bacterial viruses—bacteriophages. As BALOs are predatory bacteria that feed upon other Gram-negative bacteria, BALOs pose an attractive potential application in eradicating virulent strains of *F. nucleatum*. The current research serves to shed more light on the effect of different environmental conditions during BALO-*F. nucleatum* co-incubation, and the range of *F. nucleatum* which BALOs may predate. *F. nucleatum* exhibits a high degree of intraspecies variation; not all strains of *F. nucleatum* are virulent. As such, investigating the predatory-prey relationship between BALOs and *F. nucleatum* must include the range of potential *Fusobacterium* prey strains. Additionally, the anaerobic environment of *F. nucleatum* is not propitious for aerobic BALOs. As such, predation co-bacterial incubation assays must also be optimized. This exploratory investigation will further elucidate the potential for BALO-based targeting of virulent human-associated *F. nucleatum* strains.

Funding: Canadian Cancer Society CCSRI, Cancer Research UK, CIHR Canada Graduate Scholarship – Masters Award

10:45-11:30 Second Morning Session

Analysis of SNARE Regulation During Tumor Cell Invasion

M. Brasher[1], D. Martynowicz[1], O. Grafinger[1], M. Marchment[1], R. Shannon[1], A. Hucik[1], E. Shanks-Skinner[1] and M. Coppolino[1].
[1]Department of Molecular and Cellular Biology, Biological Sciences, University of Guelph

Tumor cell invasion involves targeted localization of proteins required for interactions with the extracellular matrix and for proteolysis. The localization of many proteins during these cell-extracellular matrix interactions relies on membrane trafficking mediated in part by SNAREs. The SNARE protein syntaxin4 (Stx4) is involved in the formation of invasive structures called invadopodia; however, it is unclear how Stx4 function is regulated during tumor cell invasion. Munc18c is a known regulator of Stx4 activity, and here we show that Munc18c is required for stx4-mediated invadopodium formation and invasion. Biochemical and microscopic analyses revealed a physical association between Munc18c and Stx4, which was enhanced during invadopodium formation. It was also found that an N-terminal Stx4-derived peptide associates with Munc18c and inhibits endogenous interactions of Stx4 with SNAP23 and VAMP2. Furthermore, expression of the Stx4 N-terminal peptide, which consists of residues 1-29 of Stx4, decreased invadopodium formation and cell invasion *in vitro*. A smaller peptide that consisted of residues 1-15 of Stx4 was also found to decrease invadopodium formation, cell migration and cell invasion. Of note, cells expressing the Stx4 N-terminal peptide 1-29 residues exhibited impaired trafficking of membrane type 1 matrix metalloproteinase (MT1-MMP) and EGF receptor (EGFR) to the cell surface during invadopodium formation. Further work was done to determine VAMP2's role during invadopodium formation. In both VAMP2 knockdown experiments and inhibition experiments, invadopodium formation and local cell invasion was decreased. This work further advances our understanding of the role of SNARE function in the localization of proteins that drive tumor cell invasion.
Funding: CRS and NSERC

Characterization of extracellular vesicles obtained from cultured explants of canine osteosarcoma and normal bone

Alicia M. Viloria-Petit[1*], Mackenzie Wong[1], Anita K. Luu[1], Michelle Oblak[2], Brigitte Brisson[2], Geoffrey Wood[3]
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[2]Department of Clinical Studies, Ontario Veterinary College, University of Guelph
[3]Department of Pathobiology, Ontario Veterinary College, University of Guelph

Osteosarcoma (OSA) is the most common bone tumour in dogs and humans. OSA often metastasizes to the lungs in both species, and this is responsible for patient mortality. There are no reliable biomarkers to predict metastatic relapse in OSA. Extracellular vesicles (EVs) are release by tumour cells into blood circulation and contain cargo that reflects their cell of origin. The goal of this research was to develop an explant culture protocol to isolate and characterize EVs and their protein cargo in canine OSA tumour tissue, for subsequent identification of prognostic/predictive signatures that could be assessed non-invasively in plasma of OSA patients.

Tumour and normal bone (NB) samples were obtained from canine OSA patients. Tissue was processed and incubated in culture media containing 5% EV-free FBS and antibiotics under standard conditions. After 24 hours, media was recovered, centrifuged, and stored. EVs were isolated from media via size exclusion chromatography, and next characterized via immunoblotting, transmission electron microscopy (TEM), and particle tracking analysis (PTA). Protein cargo was assessed by ultra high performance liquid chromatography tandem mass spectrometry (uHPLC MS/MS). EVs were shown to express flotillin and/or CD63 and PTA showed different size distribution in OSA versus NB, suggesting different EV populations. TEM revealed a predominance of cup-shaped EVs of 50-200 nm diameter in OSA samples. MS analysis identified 354 distinct proteins in OSA as compared to NB EVs. OSA EVs were enriched in proteins involved in protein translation, a number of which were reported to drive OSA progression. Our results indicate that tissue explant cultures are useful tools for the identification of prognostic/predictive EV signatures in canine OSA.

Funding: OVC Pet Trust, OVC Summer Assistantship, University of Guelph Graduate Tuition Scholarship, OGS.

Lymphoma and Symmetric Dimethylarginine (SDMA) Concentration in Dogs

A. Abrams-Ogg[1], Bronwyn Rutland[2], Phillippe Levis[2], Vicky Sabine[3], Kaya Skowronski[3], Allison Majeed[3], Dorothee Bienzle[4], Alex Zur Linden[3], Danielle Richardson[5], Anthony Mutsaers[6], Paul Woods[3]
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SDMA and creatinine were measured in 52 dogs with untreated LSA, LSA in CR or PR following CHOP chemotherapy, and LSA in relapse. Mean, median and age range were 8, 7 and 3-13 years, respectively. Fifty dogs had WHO Stage III-V multicentric nodal LSA, one dog had mediastinal LSA, and one dog had bilateral renal LSA. No dog had clinical, clinicopathologic or sonographic signs of pre-existing kidney disease when diagnosed with LSA. Results are:

Status	N	SDMA mean, median (range) [% > RI 0-14 ug/dL]	Creatinine mean, median (range) [% > RI 44-133 umol/L]	SDMA:Creatinine mean, median (range)
Untreated	36	18, 16 (5 – 90) [72%]	97, 81 (47 – 313) [12%]	0.21, 0.19 (0.06 – 0.53)

CR	19	10, 11 (4 – 18) [11%]	90, 79 (24 – 169) [17%]	0.12, 0.11 (0.06 – 0.24)
PR	6	16, 16 (14 – 19) [83%]	90, 78 (65 – 152) [17%]	0.22, 0.20 (0.14 – 0.35)
Relapse	5	27, 21 (14 – 39) [80%]	129, 105 (71 – 216) [40%]	0.21, 0.20 (0.15 – 0.27)

Comparing dogs with CR and all other dogs, differences between mean and median SDMA levels and mean and median SDMA:Creatinine were significant ($P < 0.001$), while differences between mean and median creatinine levels were not. Dogs with LSA may have increased SDMA which may normalize if dogs achieve CR, thus SDMA should not be interpreted in these dogs as evidence of age-related chronic kidney disease. SDMA might have potential as a biomarker of treatment response, although data are insufficient for conclusions in this regard.

Funding: Private Donations, Pet Trust

1:55-2:40 Afternoon Session

Targeting Mitochondrial Metabolism with Avocatin-B Induces Selective Acute Myeloid Leukemia Death

M. Tcheng[1], K. Rea [2], T. Akhtar[2], V. Cunha[3], AW Mohsen[4], J. Vockley[4], PA Spagnuolo[1]*
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[2]Department of Molecular and Cellular Biology, College of Biological Sciences, University of Guelph
[3]Department of Chemistry, Faculty of Science, University of Alberta
[4]Department of Medical Genetics, Center for Rare Disease Therapy, University of Pittsburgh Medical Center

Acute myeloid leukemia (AML) is an aggressive hematological malignancy characterized by high relapse and low survival rates, driven by cancer cell resistance to induction therapy, the primary anti-AML regimen. Unlike normal hematopoietic stem cells, the leukemic population exhibits an altered mitochondrial phenotype characterized by increased dependence on fatty acid oxidation (FAO), marking the leukemic mitochondria as an attractive pharmacological target. Previously, avocatin-B, a mixture of two avocado-derived fatty alcohols avocadyne and avocadene, was shown to targeted mitochondrial respiration to induce selective leukemic apoptosis. In the current project, avocadyne (AYNE), a long chain, odd numbered, acetylenic fatty alcohol, was the most potent FAO inhibitor towards AML cell lines and patient samples, while sparing healthy donor samples. AYNE alone showed potent anti-AML activity in vivo, significantly reducing the amount of leukemic cells in xenograft studies. The odd numbered carbon chain, terminal triple bond, and stereochemistry of the hydroxyl groups were critical AYNE's ability to directly inhibit FAO, hinder mitochondrial respiration, and induce selective leukemic death. The next objective will elucidate AYNE's molecular target; AYNE resistant cell lines will be characterized by proteomics and immunoblotting to determine the altered expression of FAO enzymes. Lentiviral knockdown of intramitochondrial FAO enzymes with altered expression, co-immunoprecipitation of intramitochondrial FAO enzymes from treated leukemic cells, and kinetic assays will determine AYNE's molecular target. Completion of these objectives is critical to our long term goal of developing AYNE into a novel clinical therapeutic that exploits the altered mitochondrial metabolism specific to AML.

Funding Source: Queen Elizabeth II Graduate Scholarship in Science and Technology (QEII-GSST)

Diet Type and Supplement Use in Healthy Dogs and Dogs with Cancer

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Nutritional supplements are commonly fed to dogs with cancer, many report doing so because of a cancer diagnosis, however no study has compared nutritional supplement use between dogs with cancer and healthy dogs. The aim of this study is to determine if differences exist in the diet/supplement types fed to dogs with cancer and healthy dogs. An online survey containing 51 questions was administered using the Qualtrics Research Suite (Qualtrics©, Provo, Utah, USA). It was distributed among clients at the Ontario Veterinary College and through social media. Data was analysed in SPSS (IBM© SPSS Statistics for Macintosh, Version 25). Chi-squares and odds ratios were used to compare categorical data. 353 surveys were analyzed. Commercial dry food was the most frequently reported diet fed to both groups but owners of healthy dogs (n=221) were four times more likely to feed commercial dry food compared to owners of dogs with cancer (n=132) ($p=2.37x10^{-9}$). Owners of dogs with cancer were two times more likely to feed a homemade raw diet (p=0.0121) and four times more likely to feed a homemade cooked diet ($p=3.35x10^{-8}$). A variety of supplements were reported, such as multivitamin/minerals, cannabidiol (CBD) oil, turmeric/curcumin, and mushroom supplements. Owners of dogs with cancer were five times more likely to feed CBD oil ($p=1.04x10^{-4}$), seven times more likely to feed turmeric/curcumin ($p=1.18x10^{-4}$) and 19 times more likely to feed mushroom supplements ($p=2.17x10^{-7}$). These results suggest owners of dogs with cancer make different dietary decisions for their dogs compared to owners of healthy dogs.

Funding: OVC Scholarship, OGS Scholarship

Effects of cannabinoids and marine oil derivatives on cell migration and tumour formation in colorectal cancer models

J. Fux[1], J.Blay[1,2]
[1]School of Pharmacy, Faculty of Science, University of Waterloo
[2]Department of Pathology, School of Medicine, Dalhousie University

The cannabis plant is a rich source of bioactives that has been inadequately studied for medical research purposes due to its past status as the source of a controlled substance. That changed in 2018 with the legalization of cannabis in Canada for both medical and recreational use and increased attention in Canadian industry for the potential of formulations that might be used for purposes of health support. In particular, the non-psychoactive cannabis constituent cannabidiol (CBD) is of great interest for possible use in circumstances that involve altered activities in host defence by disease changes in the immune and inflammatory systems. Since 2017 we have been engaged in research to examine the potential for cannabinoids to alter the behaviour of cancer cells in a way that will benefit patients at risk of or during the progression of cancer. There have been indications that certain cannabinoids may have anticancer activities, but although cannabinoid-based synthetics seem to have potential for direct killing, natural cannabinoids have been shown not to have a significant ability for cytotoxicity. Given that cancer cells acquire or 'hijack' many

of the pathways used by cells of the immune system, metastatic change is a logical direction to explore for thee natural cannabinoids. We have examined the potential for CBD and other cannabinoids to alter cell migration and homing pathways, which in cancer form the basis of its spread or metastasis. We describe here some of our recent findings and approaches using preclinical models including transwell-based migration and invasion assays and a model of extravasation and metastasis based upon the chick embryo vasculature. We report our results following exposure of colorectal carcinoma cells either to CBD alone or in combination with products derived from marine oils, particularly those from algae, which may have unique abilities in this context. This research will build on our scientific understanding of the potential benefits of oils extracted from cannabis and algae, particularly in the context of neoplastic disease, and may help add to the economic impact of cannabis deregulation in Canada by enabling progress through industry.

Funding: CanaQuest Medical Corporation Cancer Research Program**.**

LUNCH & POSTER SESSION 12:20-1:30

OVC LLC Room 1707 B & C

Posters will be displayed all day; authors please attend your posters from at least 12:45-1:15. Judges for the Poster Competition will be evaluating posters during this time.

POSTER ABSTRACTS

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1) Investigating the effects of Hyperthermia and Heat Shock Protein 70 on microRNA biogenesis

L. Abou Zeid[1], R. Mosser[1]

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Heat shock, a form proteotoxic stress, is a major trigger of apoptosis. Molecular chaperones, such as Heat Shock proteins 70 allow for the refolding of misfolded proteins, maintaining cellular proteostasis. Additionally, HSP70 allows for cell survival following hyperthermia through preventing the activation of caspases, which are proteases responsible for initiating apoptosis. Along with disrupting proteostasis, hyperthermia can also influence gene expression through altering microRNA (miRNA) levels and expression patterns. These small non-coding RNA can silence the expression of target genes through interacting with their target mRNAs, leading to their translational repression. MiRNAs are generated through a series of processing steps. The primary miRNA transcript (pri-miRNA) is processed into a shorter precursor miRNA transcript (pre-miRNA) by the ribonuclease Drosha and its partner DGCR8. The pre-miRNA is then converted to the mature miRNA species by the ribonuclease Dicer and its binding protein, TRBP. The goal of my research is to examine the effect of hyperthermia on miRNA processing and the potential role of HSP70 in protecting the miRNA processing machinery in stressed cells. We were able to demonstrate a disruptive effect of heat stress on miRNA processing as it resulted in the caspase-mediated cleavage and degradation of Drosha, DGCR8, Dicer and TRBP. We also observed a protective effect of HSP70 on miRNA processing under proteotoxic stress, as it was able to prevent the cleavage of the miRNA processing proteins. We are currently investigating the effect of hyperthermia on the function of the core miRNA processing proteins and the resulting alterations in miRNA processing.

Funding: Natural Sciences and Engineering Research Council of Canada Discovery Grant

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2) Low oxygen influences the composition of human ribosomes and alternative splicing of ribosomal protein genes

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Ribosomes are often considered tightly regulated and static in composition due to their essential role of catalyzing protein synthesis. This view is changing, as mutations in certain ribosomal proteins are tolerated by cells, albeit with disease phenotypes known as "ribosomopathies". Additionally, specialized ribosomes have been observed in stressed bacteria and yeast cells which possess transcript specificity during translation. Here, we show that the ribosomal protein complement of human ribosomes is influenced by low oxygen (hypoxia), a key feature of the tumor microenvironment. We quantified ribosomal protein levels in actively translating ribosomes by Tandem Mass Tags mass spectrometry. Our data suggest that human ribosomes are more likely to incorporate three proteins (RPL8, RPL27A, and RPL7L1) and over two-fold less likely to incorporate RPS12 in hypoxia compared to normoxia. Furthermore, hypoxia affected the expression of 20% of ribosomal protein genes and induced five alternative splicing events within a subset of these genes. We propose that an alternative splicing event within *RPS24* could act as a hypoxic tumor biomarker based on splicing trends observed in spheroids, *in vitro* models of tumor hypoxia, and human prostate tumor samples. This alternative splicing event within RPS24 produces protein isoforms with different C-termini, so current studies include elucidating the mechanism of induction of this splicing event in spheroids and the functional implications of the alternative protein isoforms in specialized translation and the cancer phenotype. This study highlights the adaptability of a fundamental biological process in human cells under low oxygen and other cellular stressors present in tumors.

Funding Sources: NSERC, Government of Ontario

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3) Canine osteosarcoma plasma microRNA profile pre- and post-amputation

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Osteosarcoma is the most common primary bone tumor in both humans and dogs. The standard of care for canine appendicular osteosarcoma involves amputation of the limb and adjuvant chemotherapy. Although the median survival time is less than a year, patient's treatment outcome is hard to predict. There is currently no decisive method to determine which dogs will benefit the most from this aggressive treatment. Therefore, it is necessary to discover and validate biomarkers that can predict clinical outcome. MicroRNAs (miRNAs) are small non-coding RNAs that are involved in numerous cell processes and have potential as biomarkers. miRNAs are present in plasma, providing easy collection by blood sampling. This study aims to profile plasma miRNA expression in healthy dogs versus dogs with osteosarcoma, both before and after amputation. By examining matched pre- and post-amputation samples from the same individuals we hope to determine which circulating miRNAs are potentially associated with the primary tumor. Plasma samples of five dogs for each group were collected and pooled, miRNA was extracted, and reverse transcribed to cDNA. Quantitative real-time PCR was conducted to determine miRNA expression

using a miRNA Array featuring 277 canine miRNAs. The miRNAs of interest from these findings were then selected for a custom miRNA array (47 miRNAs + controls) and are being used to examine correlations to clinical outcome in over 15 control and 30 osteosarcoma cases. Because miRNAs are highly conserved across species, we anticipate that miRNAs with clinical utility in our study may also benefit human osteosarcoma patients.

Funding: Pet Trust, OVC MSc Scholarship, Graduate Excellence Entrance Scholarship

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4) Investigating the role of Nck cytoskeletal adaptors in mammary development and breast cancer

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The adaptor proteins Nck1 and Nck2 are well established signaling nodes in cellular actin cytoskeleton remodeling. Although they were first identified as oncogenes over 25 years ago, there is scarce *in vivo* evidence supporting their ability to induce tumour development or metastasis. Our lab has recently shown that Nck promotes endothelial cell migration, angiogenic remodeling, and epithelial-to-mesenchymal transition (EMT), and others have reported a requirement for Nck in invadopodia formation. These processes are all correlated with invasion and metastasis of breast cancer cells. Accordingly, we have now determined that Nck1 and Nck2 are novel regulators of breast cancer progression, as well as mammary gland morphogenesis. Systemic loss of Nck1 or Nck2 produces defects, at different developmental stages, in mammary gland duct outgrowth, branching area, and terminal end buds. Furthermore, we have found that Nck1 and Nck2 are both upregulated in aggressive human breast cancers, including HER2+ and triple negative subtypes. Using the MMTV-NIC transgenic mouse model of breast cancer, which allows simultaneous expression of activated HER2/ErbB2 and Cre recombinase in mammary epithelial cells, we have shown that deletion of both Nck1 and Nck2 results in a small but significant delay in tumour onset and a profound reduction in metastasis. Protein analysis of tumours lacking Nck1 and Nck2 shows alterations in focal adhesion signaling dynamics. These findings provide new physiological insights verifying the role of Nck as an oncogene, and they reveal its potential as a target to inhibit breast cancer.

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5) β1 Integrin Mediates the Phosphorylation of MT1-MMP on Cytoplasmic Threonine-567 to Induce Tumour Cell Invasion

Olivia Grafinger[1]*, Genya Gorshtein[1], Marc Coppolino[1]
[1]Department of Molecular & Cellular Biology, University of Guelph

The majority of all cancer-related deaths occur as a result of metastasis – the dissemination of primary tumour cells through the body, resulting in the establishment of secondary tumours. In order for primary cancer cells to migrate they must invade the dense protein-rich extracellular matrix (ECM) which surrounds them. Many invasive cancer cells produce membrane protrusions,

known as invadopodia, which extend into the ECM and facilitate its degradation through their enrichment in proteolytic enzymes. It has been found that digestion of the ECM is accomplished primarily by the cell surface enzyme membrane type-1 matrix metalloproteinase (MT1-MMP), allowing tunnels to be formed through which cells can navigate. Recently, it was determined that MT1-MMP must be internalized from the plasma membrane and recycled to the migration front for a cell to maintain its invasive phenotype. Endocytosis of the enzyme is dependent on a phosphorylation event on its cytoplasmic domain, and we have previously found that β1 integrin activation using a specific antibody results in MT1-MMP phosphorylation. Through the use of nonphosphorylable mutant constructs, we show that MT1-MMP is phosphorylated on cytoplasmic Threonine-567 downstream of β1 integrin activation. Further analyses suggest that invadopodia formation and local cellular invasion downstream of β1-integrin-activating antibody treatment is dependent on phosphorylation of MT1-MMP on Threonine-567.

Funding: OGS, NSERC

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6) Inhibition of the copper chaperone Atox1 sensitizes human and canine osteosarcoma cells to carboplatin chemotherapy

Jordon M. Inkol[1], Andrew C. Poon[1], Anthony J. Mutsaers[1,2]
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Osteosarcoma (OSA) is the most common primary bone tumour in children and dogs. Treatment of OSA is similar regardless of species: surgical resection and (neo)adjuvant chemotherapy. Standard chemotherapy for OSA involves a platinum agent e.g. carboplatin or cisplatin. However, OSA patient survival has not increased in several decades, and survival is limited by recurrent disease and/or drug-resistant metastasis. Therefore, development of new or improved therapeutic modalities are required. Recent studies suggest the copper chaperone Atox1 plays a role in acquired platinum drug resistance by forming aggregates that prevent DNA adduct formation. This in vitro study investigated targeting the platinum efflux pathway using a small molecule Atox1 inhibitor, DC_AC50 (DC), in OSA cells.
Two canine (Abrams and D17) and two human (MG63 and HOS) OSA cell lines were evaluated. Clinically relevant doses of both carboplatin and DC decreased cancer cell viability in all cell lines. Furthermore, combination treatment resulted in a synergistic relationship (CI < 1), also at clinically relevant doses. Colony formation was also decreased at relevant doses. A significant dose-dependent shift towards early apoptosis was detected with combination treatment, compared to carboplatin or DC alone. DC treatment alone demonstrated a cytostatic effect, arresting OSA cells in S phase. DC treatment also significantly attenuated migration capacity with no effect on proliferation.
Targeting Atox1 may sensitize OSA cells to platinum chemotherapy, which may ultimately improve outcomes for chemotherapy-resistant OSA patients, as well as increase our understanding of the biological role of copper chaperones and transporters in cancer cells.

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7) The Effects of Nidogen-1 on Proliferation and Migration in Claudin-low Mammary Tumor Cells

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Breast cancer is the most common type of cancer among women, with one subset of the triple-negative subtype, claudin-low, known to be aggressive and metastatic. For invasion and metastasis to occur, cancer cells must cross basement membranes (BMs), which contain structural proteins such as laminin and collagen IV and linking proteins such as perlecan and nidogen, and colonize on distant BMs. Nidogen is a glycoprotein that makes up 2-3% of basement membranes and has two types: nidogen-1 (NID1) and nidogen-2 (NID2). There are limited studies on NID1 and cancer, with results demonstrating decreased invasiveness and metastatic capabilities in *Nid1* silenced cells of various cancer types. Through previous work, a murine cell line representative of the claudin-low subtype, known as RJ423, was developed; it demonstrated a 5000-fold increase in *Nid1* expression compared to the luminal subtypes. To test whether high *Nid1* expression contributes to the metastatic nature of claudin-low tumors, *Nid1* levels were knocked down in RJ423 cells and proliferation and migratory capabilities were assessed. Immunofluorescence using a phospho-histone-H3 antibody demonstrated that suppressing NID1 reduced RJ423 cell proliferation significantly. Additionally, apoptosis was assessed through flow cytometry to detect annexin V levels; however, no significant differences. Furthermore, invasion assays demonstrated a reduction in migration of collagen IV coated wells in NID1 suppressed cells. Currently, conditioned media experiments to assess migration are being conducted along with qPCR to asses EMT gene expression levels. Thus, this may provide a new area of NID1 targeted therapies to lessen the metastatic nature of claudin-low breast cancer.

Funding: OVC Scholarship, CIHR

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8) **Self-Emulsifying Delivery Systems for Bioactive Avocado Polyhydroxylated Fatty Alcohols**

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Avocatin B is a mixture of avocadene and avocadyne that possess novel anticancer activity by accumulating in mitochondria and selectively inducing apoptosis of leukemia and leukemia stem cells. Avocadene and avocadyne are avocado seed (Persea americana Mill.; Lauraceae) derived seventeen carbon polyhydroxylated fatty alcohols (PFAs) that are currently being used in topical cosmetic formulations for skin care products, and as food additives due to their insecticidal, antimicrobial, and spore-inhibiting properties. Formulations of avocatin B suitable for in vivo delivery and human oral consumption have not previously been described. We exploited the natural surface active properties of avocadene and avocadyne to design self-emulsifying drug

delivery systems (SEDDS) that employ molecular self-assembly to form fine oil-in-water (O/W) microemulsion droplet structures at the nanometer scale. Unlike typical emulsion based delivery systems, the described compositions in this work were prepared without mechanical homogenization using only one surfactant which provides significant advantages of i) a low weight ratio of emulsifying component to oil component, and ii) fewer chemical toxicity concerns. *In vitro* cytotoxicity testing of avocatin B SEDDS in acute myeloid leukemia (AML) cell lines, TEX and AML-2, indicate significant increases in potency and bioactivity compared to conventional dimethyl sulfoxide (DMSO) based delivery. A pilot pharmacokinetic evaluation of avocatin B SEDDS in C57BL/6J mice revealed appreciable accumulation in whole blood and biodistribution in key target tissues. We anticipate that data obtained from this study describe ideal delivery systems to adequately evaluate the anti-leukemic activity of avocado PFAs in future pre-clinical and clinical studies.

Funding: NSERC

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9) Investigating molecular markers using tissue microarrays for the prognosis of canine mast cell tumours

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Mast cell tumours (MCTs) are the most common skin tumour of the dog, representing approximately 21% of all skin tumours. Accurately predicting behaviour is critical in directing patient therapy in canine MCTs, as they range from benign to a fatal systemic disease. Grading is useful for prognosis, but it cannot predict the behaviour of each MCT. We hypothesized that biomarker staining in tumour tissues will correlate with patient outcome. A clinically annotated tissue microarray of skin canine MCTs (with and without adjunctive treatment) was created and high-throughput immunohistochemical staining profiling of 244 tumours from 189 dogs was performed. Mast cell tryptase levels were found to be prognostic in low-grade MCTs, with low tryptase-expressing tumours having a decreased time to recurrence and/or metastasis compared to high-tryptase expressing tumours. Two other proteins involved in protein degradation pathways were also investigated: c-CBL, an E3 ubiquitin ligase, and beclin-1, an autophagy protein. High c-CBL expressing tumours had a decreased MCT-related survival time in primary, adjunctive therapy treated, subcutaneous MCTs. Beclin-1 staining level was a strong predictive biomarker for MCTs. High beclin-1 expressing tumours showed poor response to adjunctive treatment compared to low beclin-1 expressing tumours, especially for high-grade or high mitotic count tumours. These findings will hopefully improve our ability to prognosticate MCTs and help decide whether to pursue adjunctive treatment. Importantly, this is also the first evidence that autophagy inhibitors may be useful in improving response to treatment for dogs with high-grade MCTs.

Funding: OVC Pet Trust

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10) 3TSR improves oncolytic virus therapy and metronomic chemotherapy in advanced stage epithelial ovarian cancer

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Epithelial ovarian cancer (EOC) is the leading cause of death among all gynecological malignancies. EOC lacks presentation of early symptoms and effective screening techniques, forcing diagnosis at advanced stages when treatment strategies are largely ineffective. This demonstrates the need for innovative approaches to combat advanced EOC. Oncolytic viruses (OVs) selectively lyse malignant cells and activate anti-tumor immune cells while leaving normal body cells unharmed. One difficulty with systemic delivery of OVs is that they cause vascular collapse in the irregular vessels formed by tumors, impairing subsequent viral doses and tumor perfusion of immune cells. Our lab has characterized the three type-1 repeat domains (3TSR) of Thrombospondin-1, which harness the majority of its anti-angiogenic properties. 3TSR induces vascular normalization and ovarian tumor cell apoptosis in an orthotopic, syngeneic mouse model of advanced EOC, resulting in enhanced chemotherapy uptake, regression of metastatic disease and prolonged survival. Recently, we have characterized the ability of 3TSR to combat vascular collapse resulting from intravenous delivery of an oncolytic virus. Combining 3TSR with oncolytic Newcastle Disease Virus (NDVF3aa) led to enhanced trafficking of immunological cells into both the primary tumor core as well as to metastatic lesions. Given that women with advanced EOC are treated with platinum and taxane-based chemotherapies at diagnosis, we seek to investigate the immunological effect of adding chemotherapy to our 3TSR+NDV(F3aa) combination therapy. Our data provides pre-clinical rationale to explore combining vascular normalization and oncolytic virus therapy as a treatment strategy for malignancies that typically overcome single-agent therapy.

Funding: OVC Scholarship, OGS Scholarship

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11) The Functional Utility Of A Unique Subset Of Bone Marrow-Derived Dendritic Cells For Cancer Vaccines

Robert Mould[1], Jacob P. Van Vloten[1], Corby Fink[4] Ashley Ross[1], Mankerat Singh[1], Anthony Mutsaers[2,3], James J. Petrik[2], Leonardo Susta[1], Geoffrey Wood[1], Sarah K. Wootton[1], Gregory Dekaban[4], Byram W. Bridle[1*] and Khalil Karimi[1*]
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The potency of dendritic cell (DC)-based vaccines as cancer biotherapies needs to be improved. DC culturing protocols expand heterogeneous populations of cells that include subsets of macrophages and DCs. When we compared the functionality of DCs differentiated from murine bone marrow in the presence of GM-CSF, we identified a subset of DCs that produce IL-12 but lack production of other inflammatory cytokines such as TNF-α. Interestingly, this population could be expanded when IL-4 was added during a particular window to the DC culture. Using flow

cytometer cell sorting, we isolated subsets within the heterogenous cultures and vaccinated mice with these cells after they were stimulated with lipopolysaccharide and pulsed with SIINFEKL ($OVA_{257-264}$) peptide. The IL-12 single producing subset of DCs outperformed all other subsets by inducing the highest-magnitude SIINFEKL-specific $CD8^+$ T cell responses. LPS-stimulated DCs were phenotypically characterized 12-hours post-stimulation using flow cytometry. We confirmed that not only the introduction, but also the timing of adding IL-4 into a DC culture was critical for the expansion of the unique DC population. Furthermore, we were able to demonstrate the existence of this subset in a very different culture protocol utilized by another lab, suggesting this uniquely potent subset likely exists within many commonly used culturing methods. Notably, isolation of our unique DC subset facilitated induction of high magnitude T cell responses at lower doses than conventional mixed cell vaccines, which may help alleviate manufacturing burdens. Future studies will determine why this unique subset is superior to other subsets.

Research and stipend funding provided by: Terry Fox Research Institute and Art Rouse Cancer Biology Graduate Stipend

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12) Inhibition of the Mevalonate Pathway with Simvastatin in Transformed Fallopian Tube Epithelial Cells as a Novel Therapy in Epithelial Ovarian Cancer

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The 5-year survival rate for high-grade epithelial ovarian cancer (EOC) at <30% clearly indicates an immediate need for new and innovative therapies. We have discovered that metastatic tumour cells derived from the abdominal ascites (28-2 cells) in a murine EOC model acquired a gain-of-function p53 mutation and displayed significant upregulation of the mevalonate pathway. Enhanced mevalonate signaling provided a survival advantage to 28-2 cells such that they are uniquely sensitive to inhibition of the rate-limiting enzyme of this pathway, HMG CoA reductase, by simvastatin. As EOC originates from the distal fallopian tube epithelium (FTE), and acquisition of p53 mutation is thought to be an initiating step, we hypothesize that transformed FTE cells will be addicted to mevalonate signaling and treatment with simvastatin will inhibit this pathway to induce disease regression. We have developed an orthotopic syngeneic model of early-stage EOC, with introduction of immortalized murine FTE cells into the distal fallopian tube. We will evaluate the role of p53 mutation and mevalonate signaling in reprogramming FTE cells within the oviductal microenvironment and their role in initiating high-grade disease. Preliminary *in vitro* studies demonstrated that treatment with simvastatin significantly reduced cell viability and increased apoptosis in murine FTE cell lines harbouring a p53 mutation. Understanding the mechanistic relationship between the acquisition of a p53 mutation and mevalonate pathway upregulation may provide novel therapeutic avenues including the use of simvastatin to target tumour initiating cells. Mevalonate pathway inhibition targets cancer cell metabolism and could also reduce the burden of metastatic abdominal disease.

Funding Source: OVC Scholarship, Cancer Research Society

13) Cadherin-22 signaling as a modulator of cellular adhesion and migration in hypoxic cancer cells

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Cell-cell adhesion is facilitated by adherens junctions in epithelial and endothelial tissues. These contacts enable cells to collectively proliferate and migrate in processes such as embryonic development, wound healing, and cancer progression. Cell migration and invasion are driven by hypoxia, which arises in tumors through oncogene-driven proliferation of cancer cells in the absence of efficient vasculature. Metastatic tumor cells have been shown to collectively migrate and invade into nearby and distal tissues. How this is accomplished in hypoxic cancer cells is as of yet largely unexplored. Cadherins are a superfamily of transmembrane proteins that mediate cell-cell adhesion. Our lab has identified cadherin-22 as a hypoxia-induced cell-cell adhesion protein that promotes glioblastoma and breast cancer cell-cell adhesion and invasion. Importantly, cadherin-22 colocalizes with tumor hypoxia and correlates with low patient survival in glioma and invasive ductal breast carcinoma patient tumor specimens. Here, I investigate the signaling mechanisms utilized by cadherin-22 to develop its potential as a therapeutic target against breast cancer metastasis. I will begin by investigating the relationship between cadherin-22 and of β -catenin, a molecule known to be involved in the canonical Wnt signaling pathway. I hypothesize that hypoxic induction of cadherin-22 sequesters active β-catenin to manipulate cell adhesion. Thus far, I have demonstrated evidence for cadherin-22- β-catenin interaction via co-immunoprecipitation as well as preliminary evidence for differential β-catenin activity in response cadherin-22 expression levels as demonstrated by qRT-PCR data. Moving forward, I plan to perform *in vitro* cell adhesion and migration assays, and measure β-catenin nuclear activity via immunofluorescence.

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14) Effect of Rapamycin on Canine Mast Cell Cancer Cell DNA Damage Responses Following Radiation

M. Phan[1], C. Ayoub[1], V. Poirier[2], B. Coomber*[1]
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The mTOR pathway, highly activated in cancer cells, regulates several essential cell functions. Inhibition of mTOR can be achieved using Rapamycin, a cytostatic compound demonstrating radiosensitizing effects. We examined this in two canine mast cell cancer cell lines (MCT-1, MCT-2) derived from naturally occurring tumours in pet dogs. Experiments were performed to understand how altered mTOR signalling affects DNA strand break damage response. Preliminary work revealed that Rapamycin pre-treatment led to sustained DNA damage foci after radiation compared to untreated cells. Here we examine the kinetics of this response. MCT-1 cells were pre-treated with Rapamycin for 24 or 48 hours before receiving radiation. MCT-1 and MCT-2 cells were treated with Rapamycin for 4 or 7 days post-radiation. Rapamycin doses were 50%, 100% and 150% of the plasma steady-state levels reported in canines: 5.5 nM, 11 nM and 16.5 nM respectively. Each Rapamycin dose was combined with 3 radiation doses: 3 Gy, 6 Gy and 10 Gy.

Clonogenic survival of MCT-1 decreased after 4 and 7 days of Rapamycin treatment following 10 Gy. Western blots demonstrated a dose-dependent activation of S6K in MCT-1, while MCT-2 demonstrated a higher level of sensitivity. Radiation activates mTOR in MCT-1 in a dose-dependent manner, possibly by RNF168 upregulation. Preliminary results from the comet assay suggests that mTOR inhibition sustains DNA strand breaks following radiation. Rapamycin potentially radiosensitizes canine mast cell cancer. Furthering our understanding of altering cell signalling to enhance DNA damage in cancer provides insight into clinical applications and therapeutic avenues.

Funding Source: OVC Pet Trust

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15) Validating a semi-quantitative assessment method for degree of methylene blue staining in canine sentinel lymph nodes

A. Ram[1], M. Oblak[1]
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Sentinel lymph node (SLN) mapping provides important prognostic information for metastasis. Methylene blue (MB) is the most common contrast agent used to identify SLNs, typically in combination with another agent. One of the challenges with the use of MB is that it can be difficult to discern whether a lymph node is stained blue, or if the discolouration is due to natural lymph node coloration, as brown can often appear blue. In addition, the literature does not report an objective means to score the degree of SLN staining, therefore making it very difficult to compare data between studies. While ideally, a digital algorithm would be used in every case, this is not practical in many situations. Therefore, a more objective, simple method for MB stain quantification is necessary to improve reporting. The purpose of this prospective pilot study was to develop a digital algorithm and validate a semi-quantitative scoring method for surface MB staining in whole lymph nodes. LN were assessed *ex vivo*, photographed and scored based on surface staining (0 – no blue stain, 1 – 1-50% stained, 2 – 51-100% stained). The lymph node images were analyzed for signal-to-background ratio in Image J (using a threshold of 0-125). Twelve lymph nodes were included. Scoring and analysis of lymph nodes depicted strong agreement between the semi-quantitative scoring and image analysis (K = 0.875). Further analysis will be performed to include interobserver variability and agreement between *ex vivo* and photographic scores. Based on this preliminary work, the use of a semi-quantitative scoring system shows promise for an objective assessment for MB staining in clinics and future research.

Funding: OVC Pet Trust

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16) Branched Chain Amino Acid Transaminase 1 in Claudin-low Breast Cancer

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Breast cancer, the most commonly diagnosed cancer in women, can be classified into five distinct subtypes. One subtype, claudin-low breast cancer, accounts for approximately 7% of the breast cancer cases and these tumors are notoriously aggressive. RNA sequencing of human claudin-low breast cancers by other groups and RNA sequencing of a murine claudin-low mammary tumor cell line by our group has revealed that *Bcat1* is significantly up-regulated in this breast cancer subtype. *Bcat1* regulates the metabolism of branched chain amino acids and has been linked to numerous pathologies. Based on this data we hypothesized that the expression of *Bcat1* in claudin-low mammary tumors is driving the aggressive nature of this cancer subtype and disrupting *Bcat1* will deter these features. Elevated expression of *Bcat1* in the murine claudin-low cell line RJ423, compared to the murine luminal mammary tumor cell line RJ345, has been confirmed at the mRNA and protein level. *Bcat1* has been transiently down-regulated ~70% in RJ423 cells using siRNA and this suppression of *Bcat1*, contrary to the anticipated result, showed no effect on proliferation based on phospho-histone H3 immunofluorescence or cell survival based on Annexin V staining. Cell cycle analysis using Bromodeoxyuridine and 7-AAD by flow cytometry was completed, however, no significant difference was observed. Further study analyzing the metabolic functions of *Bcat1* in claudin-low breast cancer is currently underway. This study will determine whether further investigation into the effects of *Bcat1* on claudin-low human breast cancer is prudent and if *Bcat1* may be used as a therapeutic target.

Funding: CIHR, OVC Scholarship

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17) Targeting of Mitochondrial Bioenergetics by Shikonin as a Treatment for Acute Myeloid Leukemia

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Acute myeloid leukemia (AML) is a hematopoietic malignancy that results from the accumulation of undifferentiated or poorly differentiated myeloid cells in the peripheral blood and bone marrow. Current AML therapeutics are sub-optimal and contribute to a 5-year survival rate of only 24%. AML has been characterized as having an altered metabolism, that contributes to its maintenance and growth. These alterations distinguish AML cells from their unique normal hematopoietic counterparts and present as possible targets for selective therapies. Considering this, we sought to identify novel anti-AML compounds with potential for metabolic targeting. Through a high-throughput screen of a nutraceutical library, we found that shikonin, a naphthoquinone, was a potent inhibitor of leukemic proliferation. Shikonin induced cytotoxicity in a panel of leukemic cell lines and preferentially targeted the clonogenic growth of primary leukemia cells while sparing normal hematopoietic progenitor cells. Cytotoxicity of shikonin can likely be attributed to an inhibition of mitochondrial respiration leading to a decrease in ATP production and thereafter,

energy depletion of the cell. This reduction in mitochondrial respiration was further assessed by analysis of mitochondrial electron transport chain activities where it was found that shikonin inhibited activity of complex II. Additionally, cells lacking complex II activity were less sensitive to shikonin treatment. In an *in vivo* engraftment model of AML, shikonin significantly reduced the engraftment of primary AML cells in the bone marrow and was well-tolerated. Together, these results highlight shikonin's ability to selectively target AML and warrant the further investigation of shikonin as an electron transport chain-targeting agent.

Funding: Stem Cell Network

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18) ICCI comparative oncology program: Utilizing spontaneous companion animal cancers in clinical research studies as models for human cancers

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Similar to people, cancer is common in companion animals (CA) with ~1:3 dogs and 1:7 cats developing cancer and ~50% of pets >10 years old dying of the disease. Many CA cancers share similar characteristics to human cancer types and studies in CA cancer patients enable valuable clinical data to be obtained for translational research relevant to human cancer as well as benefiting veterinary patients e.g. novel techniques and treatment options. Oncology-related clinical research trials at OVC HSC are performed with the Institute for Comparative Cancer Investigation (ICCI). The ICCI can assist with the administrative aspects of OVC HSC-based CA clinical research projects, including paperwork requirements, recruiting patients, obtaining consent, liaising with referring veterinarians, publicity & facilitating sample collection. Furthermore, the ICCI was the first Canadian member in the National Institute of Health-National Cancer Institute (NIH-NCI) Comparative Oncology Trials Consortium (COTC). Since 2014, over 1100 patients have been recruited into 35 oncology-related studies (many OVC Pet Trust funded) at the OVC HSC. Currently there are 11 studies recruiting oncology patients: 9 canine, 1 feline and 1 both species (http://ovc.uoguelph.ca/icci/trials) and another 5 are closed for recruitment but are still actively monitoring and collecting samples from recruited patients. Three studies (2 closed & 1 open) are collaborations with COTC, all investigating osteosarcoma in dogs, results of which are of particular importance for pediatric osteosarcoma. The ICCI comparative oncology program has the potential to not only facilitate the improvement of healthcare and the lives of CA, but also those for humans.

Funding Source: OVC Pet Trust and The Smiling Blue Skies Cancer Fund.

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19) The ICCI Companion Animal Tumour Sample Bank: facilitating translational cancer research

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The Companion Animal Tumour Sample Bank (CATSB) continues to successfully facilitate basic and translational veterinary oncology research. Currently, CATSB has over 1300 cases banked and has contributed samples to 20 intramural and extramural research projects. Located in the OVC HSC Mona Campbell Centre for Animal Cancer, the CATSB is the only veterinary oncology tissue bank in Canada and is registered with the Canadian Tissue Repository Network. Sample types collected and stored at ultracold temperature are: serum, plasma, buffy coat, urine, and tissue. Tissue samples (tumour and matched normal), are collected immediately following surgical excision and are available as flash frozen, in RNAlater, and in CryoMatrix. Tumour tissue is also formalin fixed, paraffin embedded, sectioned, and H&E stained for quality control analysis by a pathologist. Prospective sampling can also be tailored to suit the needs of researchers. The three most prevalent canine tumour types are soft tissue sarcoma, lymphoma, and osteosarcoma, but a variety of other neoplasms have also been banked. There are also currently 12 primary cell lines from canine and feline tumours available, with more in development. In addition to samples, researchers can receive patient signalment, histopathology, and follow-up data. Researchers access samples by filling out a short application form. A cost-recovery fee (which is subsidized for University of Guelph researchers) is applied to enable the CATSB to continue its mission: to facilitate veterinary research to improve the lives of companion animals with cancer, with the potential to contribute to comparative human cancer research.

Funding: OVC Pet Trust and The Smiling Blue Skies Cancer Fund

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20) Off-Target Infection of Stimulated T Cells by Vesicular Stomatitis Virus Has Implications for Single- Versus Multi-Dosing Oncolytic Virotherapy Protocols

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The field of oncolytic virotherapy was founded on the premise that the dominant mechanism of action was direct virus-mediated killing of cancer cells. This resulted in a paradigm of rapid, repeated delivery of oncolytic viruses (OVs) to maximize infection of tumours before the immune system cleared the virus. However, recent research argues that induction of tumour-specific immune responses is an equally important mechanism. If OVs are to be used as *in situ* or conventional transgene-encoding vaccines, the ideal dosing regimen may need to be reconsidered. From an immunological perspective, rapid, multi-dosing protocols for vaccines are usually avoided. Further, studies have shown that activation of leukocytes can promote their infection with viruses. Therefore, we hypothesized that activated leukocytes might become susceptible to killing

by OVs, making single-dosing regimens superior in some contexts. Indeed, *in vitro* flow cytometry studies demonstrated that activated T cells were susceptible to infection with vesicular stomatitis virus (VSV), resulting in their death. Intravenous administration of VSV to mice followed two days later by a second dose showed that multi-dosing potentiated infection of T cells *in vivo*. Moreover, multi-dosing with a VSV-vectored booster vaccine at a two-day interval abrogated survival of mice with intracranial melanomas, as compared to a single-dose protocol. Further, infection of activated human blood-derived T cells was pronounced, suggesting the findings have clinical relevance. These results suggest that multi-dosing protocols might be contraindicated for some applications of OVs. Optimal dosing frequencies should be carefully evaluated before oncolytic virotherapies enter clinical trials.

Operating funds were from the National Centre of Excellence in Biotherapeutics for Cancer Treatment (BioCanRx) and the Terry Fox Research Institute (to BWB). Stipend funding was from: Vanier Canada Graduate Scholarship, Brock Doctoral Scholarship, and the Ethel Rose Charney Scholarship in the Human/Animal Bond (AAS); Canadian Institutes of Health Research Graduate Scholarship (Masters Award) and Ontario Veterinary College (OVC) Pet Trust Scholarship (AWKAY); Art Rouse Cancer Biology Graduate Stipend (RM); OVC Graduate Scholarship (TM); Ontario Graduate Scholarship (JvV).

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21) Targeted DNA sequencing of matched normal, primary, and metastatic canine appendicular osteosarcoma

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Osteosarcoma (OSA) is the most common primary bone tumor in both humans and dogs. OSA of long bones is a very aggressive cancer with the main cause of mortality being metastasis, which occurs mostly in the lungs in both species. Canine appendicular OSA is similar to human conventional OSA, but is more frequent and aggressive, making dogs a good model for the human disease. Despite the clinical importance of metastases, we know relatively little about the mutations found in these lesions, or how similar the genomes of primary tumours are to their corresponding metastases. The purpose of this study was to conduct targeted sequencing of selected cancer-related genes and compare matched normal, primary and metastases from the same patient in a cohort of 8 dogs. Each sample had 4 high-depth sequencing runs, comprised of targeted sequencing of 586 specific genes using Illumina Hi-Seq. Their reads were aligned to the dog reference genome canfam3 using BWA. Variant calling was performed, using Mutect2 and VarScan2. Variants were compared between the different groups. Varscan2 and arrayCGH were used to call copy number changes in the dogs. These calls were processed by the R library DNAcopy to predict large scale copy number aberrations. Overall, matched primary and metastatic samples (within the same patient) had more in common than samples from the same location (primary or metastasis) across different patients. The only gene which was mutated in multiple matched primary-metastatic samples was TP53. There were several genes with predicted

consequential mutations that were only found in metastases and these may be important for the phenotype of metastatic cells.

Funding: OVC Scholarship

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22) Investigating the Synergistic Interaction between Adaptor Protein ShcD and Tie2 Receptor in Glioma Cell Invasion

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Malignant gliomas are often characterized by two key features: Misregulation of growth factor receptor-mediated signaling cascades and induction of a malignant phenotype upon activation of invasion and metastatic events. Receptor tyrosine kinases, phosphatases, and adaptor proteins with modular interaction domains comprise a specialized signaling circuitry that translates extracellular signals into responses through altered states of tyrosine phosphorylation. Prototypical adaptor proteins such as the Shc (Src homology and collagen) family act as cytosolic sensors and bridge different components of signaling pathways upon upstream receptor engagement. We previously showed that ShcD, the most recently isolated member, holds oncological importance owing to its overexpression in malignant gliomas compared to their benign counterparts and also demonstrated that ShcD promotes constitutive phosphorylation of EGFR in the absence of its ligand EGF. Here, we show that ShcD interacts with, and is phosphorylated by, Tie2/TEK, an angiogenic receptor tyrosine kinase, which modulates cross-talk between glioma and endothelial cells in the tumor microenvironment. Co-immunoprecipitation experiments confirmed that this interaction is primarily mediated through the ShcD SH2 domain and Y1100 on the cytoplasmic tail of Tie2. We generated U87 (more invasive) and T98G (less invasive) glioma-derived cell lines, stably expressing ShcD and/or Tie2, to demonstrate increased invasion potential of cultured cells resulting from a synergistic interaction between the two proteins shown by functional assays such as transwell invasion and invadopodia-formation simultaneous with three-dimensional spheroid migration, and invasion assays. This study will thus offer insights into the uncharted role of ShcD as an active node in the cancer metastatic network.

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23) Quantifying T-Cell and Antibody Responses Induced by Antigen-Agnostic Immunotherapies

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Immunotherapies for cancers encompass any treatment that re-targets a patient's own immune system against their malignancy. Recent years has seen successful translation of immunotherapies to the clinic, which is highlighted by immune checkpoint blockade and oncolytic virotherapy. Research into the development and optimization of new immunotherapeutic strategies is gaining momentum. To support evaluating these strategies, researchers require methods to detect and quantify induced immune responses, specifically those governed by adaptive cytotoxic T cells and antibody-producing B cells. Until recently, most methods to evaluate these immune responses relied on prior knowledge of target antigens. However, genetic studies have shown that tumors can harbor many antigens capable of eliciting immune responses, and these can differ substantially across tumor types, between patients and even within the same tumour. This realization has led to the testing of "antigen-agnostic" immunotherapies that allow tumor-derived cells to drive the immune responses instead of pre-selecting target antigens. We have developed two independent methods without the prerequisite of defining target antigens; one to quantify tumor-specific cytotoxic and helper T cells, and one to quantify tumor-directed antibody responses. These methods allow researchers to expand their preclinical research models to those that do not have defined tumour antigens, and can be modified to monitor immune responses in veterinary and human patients receiving immunotherapies.

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24) Preliminary experience using indocyanine green for intraoperative sentinel lymph node mapping in dogs with solitary tumours

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The presence of lymphatic metastasis can significantly impact prognosis and treatment recommendations. The first lymph node that drains a primary tumour is termed 'sentinel' (SLN) but this lymph node does not follow an expected anatomic drainage pattern. Determining the SLN allows for accurate determination of metastatic status leading to more accurate tumour staging and decreased need for extensive lymph node dissection. Techniques are varied for SLN mapping but often require a radioactive tracer. Recently, near infrared fluorescence imaging (NIRF) with indocyanine green (ICG) for intraoperative SLN mapping has become standard of care in the human literature. This diagnostic procedure has gained interest in veterinary oncology; however, only a few studies are available. This report describes our preliminary experience with this technology. Sixteen dogs with various solitary tumours were included. Diluted ICG was injected in 4-quadrants around the tumour site. Using NIRF (Karl Storz VITOM ICGII camera), lymph nodes were assessed for fluorescence and histopathology performed. Primary mass sites included Oral (n=6), Lung (n=2), Mammary (n=2), Cutaneous (n=5), and subcutaneous (n=2). One dog had two cutaneous masses at separate sites. The SLN identification rate was 87.5% (14/16) with a mean of 2 SLN per patient. Thirty one percent (5/16) of cases had a metastatic SLN; however, a false negative rate of 40% was identified. Overall, the intraoperative use of NIRF and ICG results

in a high rate of SLN detection; however, based on this data, an additional diagnostic method is necessary to ensure all SLNs are identified.

Funding: Pet Trust

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25) Investigating the Role of Nck during Invadopodia Formation and Invasion in Metastatic Breast Cancer

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Despite advances in breast cancer treatment, metastatic breast cancer remains incurable, and the molecular signals that promote the invasion of cancer cells from primary tumours are poorly understood. During invasion, metastatic cancer cells form actin-based structures, called invadopodia, that protrude into and degrade the surrounding extracellular matrix (ECM). Here, we identify the Nck family of cytoskeletal adaptor proteins as novel determinants of breast cancer cell invasion. Specifically, we show that both *Nck1* and *Nck2* are prominently upregulated in aggressive breast cancer subtypes associated with metastasis, including HER2+ and triple negative subtypes. To test the role of Nck1/2 in invasive processes, stable lines of triple negative MDA-MB-231 cells overexpressing Nck1 or Nck2 were created and their ability to form invadopodia, degrade gelatin (an ECM analogue) and invade through an ECM-like matrix was quantified. 80% of the Nck1 overexpressers and 82% of the Nck2 overexpressers formed invadopodia compared to 58% of the control cells. The amount of gelatin degradation was 43.9% higher for Nck1 overexpressers and 33.2% for Nck2 overexpressers, compared to the control. In transwell invasion assays, cells overexpressing Nck1 had a 1.78-fold increase in invasion, while cells overexpressing Nck2 had a 1.72-fold increase, compared to the control. Ongoing experiments will further characterize these cell models to determine how overexpression of Nck increases invadopodia formation and invasion. A small molecule inhibitor of Nck is also being tested for its ability to reduce invasion and invadopodia formation. Overall, Nck offers a potential target for the characterization and treatment of metastatic breast cancer.

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