# European Society of Veterinary Oncology

## Proceedings

**28 – 29 May 2021**

**Virtual Congress**

European Society of
Veterinary Oncology

## SCIENTIFIC COMMITTEE

Iain Grant (ESVONC)
Irina Gramer (ESVONC)

We would like to extend our thanks to the esteemed colleagues who have helped with the abstract review process:

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## Message from the ESVONC Executive Committee

We are finally here and ready to welcome you - virtually - for your 2021 ESVONC Congress.

In 2020, being a Spring Congress, we had no other choice but to cancel and it was certainly very sad not to see each other physically, but I guess we were all busy trying to cope with the pandemic, personally and professionally. We have all witnessed what quickly happened globally with the rise of virtual platforms allowing us to carry on with day-to-day business meetings as well as online conferences. And our turn has now come!

In our case, this 2021 edition is made up of the 2020 programme with a few speaker changes. At our request, authors of abstracts accepted for our 2020 Congress were given the opportunity to present at VCS instead (fast tracked to an International Session - Thank you VCS!!) or remain in the ESVONC programme for 2021. Many new submissions were also received this year and reviewed by our scientific committee, and this year's selection of posters and oral presentations cover a wide and diverse range of topics.

We hope you enjoy this programme and we would like to remind you that all presentations are pre-recorded and the videos will be available to replay for months after the Congress.

We wish to thank you, our members, for your renewed support and for being part of ESVONC. Due to the circumstances, a reduced membership fee was agreed for everyone this year while the activities of the Society were still ongoing.

We also wish to thank our all amazing sponsors, who continue to believe in us. It is really great to see that despite everything we can continue to count on them to support our event.

We wish you all a wonderful online Congress and look forward seeing you - for real this time - next year!

Your Executive Committee - Ana, Iain, Jerome, Neil and Irina

## World Veterinary Cancer Congress

The WVCC initially scheduled for 2020 in Tokyo and organised by the Japanese VCS will possibly be postponed to 2024. More info should be available in the next few months.

The organizers of the World Veterinary Cancer Congress (VCS, ABROVET, ESVONC, JVCS and AMONCOVET) have met several times over the past year to discuss the future of the WVCC and we would like your opinion. Your responses to just a few questions will help us to determine the value and future direction of this Congress. No matter what type of member you are, including technicians, students, interns and residents, your opinion matters.

https://www.surveymonkey.com/r/HC2WJWP

IF YOU ARE A MEMBER OF MULTIPLE ORGANIZATIONS AND RECEIVE MULTIPLE OPPORTUNITIES TO PARTICIPATE, PLEASE REPLY ONLY ONE TIME.

Thank you!

The WVCC Organizing Committee

Without our sponsors it would be impossible to organise a conference like this!
On behalf of ESVONC, the Congress Committee wishes to express sincere gratitude.

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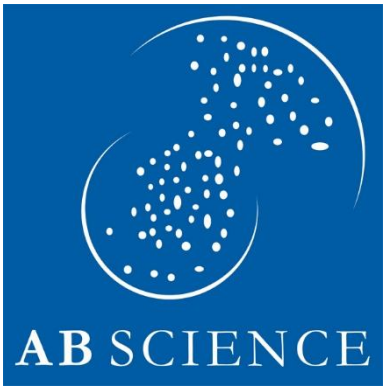


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# SCIENTIFIC PROGRAMME

**Times are CEST: UK is -1hr COLORADO TIME IS -8hrs and CALIFORNIA is -9hrs**

**Friday 28 May 2021**

| | Stream 1 | Stream 2 | |
|---|---|---|---|
| 10:00 – 10:15 | WELCOME<br>*ESVONC President Ana Lara Garcia* | | 10:00 – 10:15 |
| | Oral Abstracts | | |
| | Moderator: Joanna Morris | Moderator: Owen Davies | |
| 10:15 – 10:27 | Canine Multiple Myeloma: a review on diagnosis, staging, treatment and prognosis with evaluation of novel data<br>*H. Moberg* | The use of cannabinoids for canine medical conditions among Danish dog owners<br>*P. Holst* | 10:15 – 10:27 |
| 10:27 – 10:39 | Chemotherapy improves survival in dogs with mesothelioma: a case-control retrospective study on 37 cases<br>*M. Lajoinie* | Fluorescence-guided surgery in dogs with superficial tumors using a novel integrin-targeting near-infrared fluorescent contrast agent<br>*S. Favril* | 10:27 – 10:39 |
| 10:39 – 10:51 | Adjuvant vinorelbine in the management of pulmonary carcinoma in dogs<br>*C. Williams* | *Abstract cancelled* | 10:39 – 10:51 |
| 10:51 – 11:03 | Efficacy and tolerability of zoledronate in the treatment of dogs with hypercalcemia of malignancy or tumour associated bone pain<br>*M. Lopes* | Treatment of feline nasal planum squamous cell carcinoma (fNP-SCC) using electrochemotherapy (ECT) alone<br>*P. Simcic* | 10:51 – 11:03 |
| 11:03 – 11:15 | Evaluation of intravesical administration of carboplatin in association with nonsteroidal anti-inflammatory drugs in dogs with bladder urothelial carcinoma- retrospective study<br>*I. Rodrigues* | New potential tyrosine kinase inhibitors (TKIs) for treatment of canine mast cell tumors (MCTs)<br>*S. Gamperl* | 11:03 – 11:15 |
| 11:15 – 11:27 | Host and Viral Transcriptomic Features of Equine Sarcoids<br>*S. Jones* | Benefit of hepatic and splenic cytology in dogs with cutaneous mast cell tumors in the presence and absence of negative prognostic factors<br>*C. Fejös* | 11:15 – 11:27 |
| 11:27 – 12:00 | Q&A | Q&A | 11:27 – 12:00 |
| 12:00 – 12:30 | BREAK | Oncowaf.eu, international website on animal cancer information and clinical trials<br>*Laetitia Cicchelero, Tom Hendrickx* | 12:00 – 12:30 |
| 12:30 – 13:00 | BREAK | BREAK | 12:30 – 13:00 |
| 13:00 – 13:30 | STELFONTA – Sponsored by Virbac<br>*Pamela Jones* | | 13:00 – 13:30 |

| | Stream 1 | Stream 2 | |
|---|---|---|---|
| | Themed Session – Rescue Protocols: What to do when first line fails | Themed Session – How Advanced Imaging is and will be changing Oncology | |
| | Moderator: David Vail | Moderator: Åste Søvik | |
| 13:30 – 14:20 | Lymphoma Rescue with Cytotoxic Chemotherapy – A Cynics Perspective<br>*David Vail* | Advanced/Functional Imaging (PET/CT, PET/MR) in Companion Animal Cancer<br>*David Vail* | 13:30 – 14:20 |
| 14:20 – 14:25 | BREAK | BREAK | 14:20 – 14:55 |
| 14:25 – 15:15 | Beyond Cytotoxic Chemotherapy – Novel Approaches to Lymphoma Rescue<br>*David Vail* | Osteosarcoma. From Imaging to Therapy, and from Therapy to Imaging. Is it different between humans and dogs?<br>*Maia Vanel and Daniel Vanel* | 14:55 – 15:45 |
| 15:15 – 15:35 | BREAK | BREAK | 15:45 – 16:10 |
| 15:35 – 16:20 | Interventional Oncology – Embolization and Microwave Ablation<br>*Gerard McLauchlan* | Advanced CT and MRI in Veterinary Oncology: Present & Future<br>*Wilfried Mai* | 16:10 – 16:55 |
| 16:20 – 16:25 | BREAK | BREAK | 16:55 – 17:20 |
| 16:25 – 17:15 | Interventional radiology for oligometastatic disease and cancer palliation<br>*Lambros Tselikas* | Imaging for improved radiotherapy – planning, delivery, response monitoring<br>*Åste Søvik* | 17:20 – 18:00 |
| 17:15 – 18:00 | GROUP DISCUSSION | | |
| | | GROUP DISCUSSION | 18:00 – 18:45 |

**Saturday 29 May 2021**

| | Stream 1 | Stream 2 | |
|---|---|---|---|
| | Oral Abstracts | | |
| | Moderator: Stefano Comazzi | Moderator: Henrik Rönnberg | |
| 10:00 – 10:12 | Diagnostic performances of lymph node cytopathology in predicting lymphoma and its who histotype in dogs<br>*V. Martini* | Vitamin D status in female dogs with mammary tumours<br>*I. Lopez* | 10:00 – 10:12 |
| 10:12 – 10:24 | Interest of whole-body immuno-SPECT-CT for canine diffuse large B-cell lymphoma (DLBCL) staging before radioimmunotherapy (RIT) using an anti-CD22 monoclonal antibody<br>*C. Ibisch* | Human TNBC marker gamma Klotho is upregulated in canine mammary carcinomas: a preliminary study<br>*K. Fon Tacer* | 10:12 – 10:24 |
| 10:24 – 10:36 | WNT-genes and VEGFR -1/2 are overexpressed in T-Cell lymphomas, but do not predict treatment response<br>*M. Zandvliet* | Prognostic value of c-Myc overexpression in feline invasive mammary carcinomas<br>*F. Nguyen* | 10:24 – 10:36 |
| 10:36 – 10:48 | Retrospective study of T-cell leukaemia (large granular lymphocytes variant) in dogs associated with suspected immune-mediated cytopenias in absence of peripheral lymphocytosis<br>*A. Capasso* | Downregulation of CD146 sensitizes radio-resistant canine oral melanoma cell lines to irradiation<br>*B. Pratscher* | 10:36 – 10:48 |
| 10:48 – 11:00 | Prognosis of feline gastrointestinal small cell transmural lymphoma<br>*P. Clemente-Vicario* | A chimeric Human/Dog CSPG4 DNA vaccine reveals potential therapeutic effects against malignant melanoma<br>*F. Riccardo* | 10:48 – 11:00 |
| 11:00 – 11:12 | Differentiation between cortical and medullary origins in canine adrenal tumors using contrast-enhanced ultrasound vs plasma catecholamines vs urinary metanephrines<br>*T. Nagumo* | Q&A | 11:00 – 11:30 |
| 11:12 – 11:45 | Q&A | BREAK | 11:30 – 12:00 |
| 11:45 – 12:00 | BREAK | ESVONC Annual General Meeting | 12:00 – 13:00 |
| 13:00 – 13:30 | European Canine Lymphoma Network: An update<br>*Stefano Comazzi, Laura Marconato* | BREAK | 13:00 – 13:30 |

| | Stream 1 | Stream 2 | |
|---|---|---|---|
| | Themed Session – Endocrine Neoplasias | Resident Workshop – Angiogenesis and Cancer | |
| | Moderator: Sara Galac | Moderator: Barbara Bacci | |
| 13:30 – 14:20 | Adrenocortical tumors in dogs: building bridges between endocrinology and oncology<br>*Sara Galac* | Tyrosine-kinase receptors and their role in angiogenesis and tumour progression<br>*Barbara Kitchell* | 13:30 – 14:20 |
| 14:20 – 14:55 | BREAK | BREAK | 14:20 – 14:55 |
| 14:55 – 15:45 | Behind the mystery: understanding canine pheochromocytoma<br>*Sara Galac* | Can we effectively target angiogenesis? Available molecules, their applications and potential toxicities<br>*Barbara Kitchell* | 14:55 – 15:45 |
| 15:45 – 16:10 | BREAK | BREAK | 15:45 – 16:10 |
| 16:10 – 16:55 | The science behind canine and feline pituitary tumors: novel approaches<br>*Sara Galac* | Vessels and cancer: a complex relationship<br>*Barbara Bacci* | 16:10 – 16:55 |
| 16:55 – 17:20 | BREAK | BREAK | 16:55 – 17:20 |
| 17:20 – 18:00 | Pituitary tumors in dogs and cats<br>*Bjorn Meij* | Methods to evaluate angiogenesis and vascularization in canine and feline patients<br>*Barbara Bacci* | 17:20 – 18:00 |
| 18:00 – 18:45 | GROUP DISCUSSION | GROUP DISCUSSION | 18:00 – 18:45 |
| 18:45 – 19:00 | Closing/prize announcements/we'll meet again.. | | 18:45 – 19:00 |

# TABLE OF CONTENT

# GENERAL COMMUNICATIONS

# Oncowaf.eu, international website on animal cancer information and clinical trials

Laetitia Cicchelero[1, 2]
Tom Hendrickx[2,3]

[1] Ghent University, Belgium
[2] Belgian Cancer Fund for Animals
[3] SANIMALIA Animal Clinic, part of Evidensia Group

**Introduction**
We all have probably had the following observations:
**-Dog owners** have many questions on cancer, appreciate clinical trials and contact with people who are in the same situation.
**-Veterinarians** appreciate to have an extra option for dog owners who have tried everything or for those who are financially limited. Furthermore, they value being able to contribute to scientific progress.
-Ongoing **clinical trials** in veterinary medicine are often unknown by dog owners and veterinarians.

Furthermore, we all agree that progress in general depends on informed decisions. Unfortunately, in veterinary medicine therapeutic approaches are often based on a few trials with low statistical power (median n = 33). [1]Tan et al. suggest following reasons for this low power: a lack of awareness and/or collaboration of the public and veterinarians, limited resources or difficulty in recruiting willing owners and eligible participants. Indeed, clinical trials in Europe are typically scattered on research pages of different universities or veterinary centers, rarely visited by other veterinarians or companion animal owners.

**In comes Oncowaf**
Oncowaf.eu is an independent website created by veterinarians active in oncology to offer dog owners answers to questions they may have on cancer.
Why the name Oncowaf? Presumably, the first part is no mystery. About the waf part: there are many interpretations of the barking sound of dogs, but as Oncowaf has Belgian roots and received financial support from the Belgian Cancer Fund for Animals it was decided to choose “waf” (which is Dutch and French for “woof”). Hence: Oncowaf.
Although it received support from a Belgian Fund, the goals of the platform are not limited to the Belgian borders.

Oncowaf.eu offers detailed content on several canine oncology related topics (not limited to descriptions of tumor types, what the veterinarian can do for the cancer patient, what the owners can do to support their dog, which dog breed is more susceptible to which tumor type). Furthermore, visitors can access a searchable list of ongoing European oncology trials in the clinical trials tab. Via the Oncowaf Facebook group interested dog owners can exchange experiences and questions,
and via the Oncowaf Facebook profile owners and veterinarians remain aware of veterinary oncology information.

**Build a European clinical trial network together**
European veterinary researchers can make use of the platform offered by Oncowaf to share ongoing trials (much like AVMA's Animal Health Studies Database)[2] and thus increase awareness and recruitment on clinical trials.
The searchable clinical trials database allows you to search based on tumor type and/or country. Per ongoing trial the following minimal information is shown: a trial reference, the tumor type, the participating center(s), the in/exclusion criteria and contact details of the principal investigator(s). This listing is meant to easily find nearby trials and the means to contact the study in case of interest.

Ideally, these efforts are eventually in time merged with the AAHSD database into a single veterinary clinical trial database, an equivalent of clinicaltrials.gov in humans.

**Call to action**
We vividly encourage veterinarians to share their ongoing clinical trials and diagnostic and/or treatment options with us. The former to increase recruitment and treatment options for dogs, the latter to help dog owners to locate non-standard treatments such as photodynamic therapy, electrochemotherapy, radiotherapy etc.

You can stay up to date on ongoing clinical trials via ESVONC's communication channels. When a study is started, you will be notified.

For now, Oncowaf.eu is available in three languages: Dutch, French and English. If you would like to contribute to the translation of Oncowaf to another language, feel free to contact us at contact@oncowaf.com.

**References**
[1] Tan YJ, Crowley RJ and Ioannidis 2019. An empirical assessment of research practices across 163 clinical trials of tumor-bearing companion dogs. *Scientific Reports* **9**, 11877.
[2] AVMA's Animal Health Studies Database
https://ebusiness.avma.org/aahsd/study_search.aspx

## European Canine Lymphoma Network: An update

Stefano Comazzi, University of Milan, Italy
Laura Marconato, University of Bologna, Italy

The European Canine Lymphoma Network (ECLN) is a community of European researchers working in Academia or private practice, who are involved in basic research, diagnosis and treatment of canine lymphoma. ECLN was officially founded in 2013 in Lugano (CH) following a pilot idea of the Universities of Milan and Vienna with the specific aims to define common research targets, harmonize laboratory protocols as a pre-requisite to the validation of specific diagnostic tools, and establish effective collaborations and integrated prospective research projects among interested individuals. The Network is intended to be open not only to veterinarians, but also to biologists and clinicians from the human field interested in canine lymphoma as a model of spontaneous pathology and to pharma industries searching for a relevant model for drug development.

The Network is now linking more than 90 researchers from about 30 different institutions in Europe. Also, since 2018, ECLN has affiliated to the European Society of Veterinary Oncology (ESVONC) as a working group. A survey in order to update all members is planned in the next months.

ECLN meetings are synchronized with the International Conference on Malignant Lymphoma (ICML), held in Lugano every second year. ICML is the most important world meeting on human lymphomas. The ECLN workshop gives the opportunity to update members with ongoing studies and to set up future initiatives.

Additionally, all ECLN activities are periodically updated via Facebook page (www.facebook.com/eu.can.lymph.net), that is open to all interested researchers.

ECLN supports specific projects that are constantly proposed to the members, ultimately aimed at creating guidelines on different topics and advancing knowledge and progress.

To date, the network itself has supported 5 published research papers or review articles on top rank international journals, including the mission of ECLN, consensus documents on specific topics and one research project on breed predisposition. Also, the Network created the environment for research projects among researchers from different sites, as recently seen in some presentations at the ESVONC meetings.

In 2021, due to the COVID-19 emergency, the biannual meeting of the European Canine Lymphoma Network will be moved online. This will create the opportunity to open the workshop to a wider audience of participants worldwide and to host presentations from some of the most important researchers in the world. The meeting is scheduled on June, 25th, 2021 at 15,00h Central Europe Time and it will be managed by the University of Zurich (Switzerland). The topic of the 2021 meeting focuses on the new therapies for canine lymphoma, and three invited presentations followed by a round table on the topic is planned thereafter.

The meeting will be open to all the participants; the registration to the event will be soon available on the Facebook page and is free of charge.

All colleagues interested to participate to the network activities and to attend the meeting are invited to join the Facebook community and keep posted.

# ESVONC 2021 - Keynote Speakers

**Barbara Bacci** DVM PhD DipECVP

Barbara obtained her degree in Veterinary Medicine from the School of Veterinary Medicine AT THE University of Bologna (Italy) in 2003. From 2004 she started her training in Veterinary Pathology, obtaining her PhD in Anatomic Pathology in 2007 and the master's degree in Veterinary Public Health in 2010 (University of Bologna, Italy). In 2010 she passed the board examination for the European College of Veterinary Pathologists (ECVP). After working several years in a commercial laboratory as diagnostic histopathologist, in 2010 she gained a position as Senior Lecturer in Veterinary Anatomic Pathology at the University of Melbourne (Australia) and in 2015 she gained the same position at the University of Surrey (UK). In 2018 she moved back to Italy where she is currently employed as Associate Professor in Veterinary Pathology at the University of Bologna. Her research interests are mostly focused on digital pathology, small animal and equine oncologic pathology.

**Sara Galac** graduated from the Veterinary Faculty in Ljubljana, Slovenia After graduation she moved to the Netherlands and completed an internship and residency at the Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine in Utrecht She completed her first PhD thesis about the use of a progesterone receptor antagonist during pregnancy and luteal phase in dogs at the University in Ljubljana in 2001 and her second PhD entitled "Recent developments of Cushing`s syndrome in dogs", she successfully defended at Utrecht University in 2010. Nowadays, she is a staff member of the Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine in Utrecht and holds a position of assistant professor in Internal medicine.
Her main interest is Endocrinology. She is involved in clinical work, teaching, and research. She leads an Oncology research group OnGo at the dept. of Clinical Sciences. Her research is greatly supported by national and international grants.
Sara Galac described the two novel forms of canine Cushing`s syndrome in dogs for the first time: ectopic ACTH secretion syndrome and food-dependent hypercortisolism. She strongly believes in the value of case reports to clinical practice and medical science. Her publication list consists of journal articles, research abstracts, and book chapters. She is a frequent speaker at international veterinary conferences and much involved in veterinary continuing education. She has been a president of European Society of Veterinary Endocrinology (ESVE) and is an active member of a human

adrenal network ENS@T, which joins the world leading endocrinologists and serves to stimulate research in adrenal cancer.

**Dr. Barbara E. Kitchell** graduated from Purdue University School of Veterinary Medicine in 1979. Dr. Kitchell completed an internship at the University of Minnesota, then residencies in Small Animal Medicine and Oncology at UC Davis from 1981-1985. She started an Oncology referral practice at Special Veterinary Services, Berkeley, California in 1985, and continued to manage that practice on a full time basis until 1989, when she embarked upon her graduate studies while continuing to work part time as a clinical oncologist. She received her Ph.D. degree (emphasis in Cancer Biology) from the Department of Comparative Pathology at UC Davis in 1994. Dr. Kitchell completed a postdoctoral fellowship in the Department of Comparative Medicine, Stanford Medical School from 1990-1994. She taught clinical and comparative oncology at the University of Illinois School of Veterinary Medicine from 1994-2003, followed by a faculty position at Michigan State University as Full Professor and Director of the Center center for Comparative Oncology. She held this post until joining VCA Veterinary Care Referral Center in 2013. She is the Director of Residency Training Programs for VCA, as well as Specialty Medical Director and staff oncologist at Vet Care. Dr. Kitchell is an ACVIM Diplomate in the specialties of Internal Medicine and Oncology. She is past president of the Veterinary Cancer Society and Past Chair of the Board of Regents of the American College of Veterinary Internal Medicine. Dr. Kitchell is the author of over 100 scientific publications, abstracts, proceedings, book chapters, handbooks in her field of veterinary and comparative oncology. Like most veterinarians, she has a menagerie of dogs and cats.

**Dr. Mai** received his Dr Méd Vét from the School of Veterinary Medicine of Maisons-Alfort, France, in 1995 and after a 2-year internship in small animal internal medicine, he completed a residency training in radiology and a Master's degree in Biomedical Engineering in Lyon, France. He then completed a PhD in Biomedical Engineering in Lyon, France (Université Claude Bernard) and at Duke University, North Carolina. He became a Diplomate of the European College of Veterinary Diagnostic Imaging in 2000 and a Diplomate of the American College of Veterinary Radiology in 2006.
Dr Mai is now a Professor of Radiology at Penn Vet (University of Pennsylvania) and the chief of Radiology as well as residency director. He is the author of a new MRI textbook entitled "DIAGNOSTIC MRI IN DOGS AND CATS". Other than radiology, Dr Mai is a passionate Brazilian Jiu-Jitsu practitioner and currently a brown belt in the "gentle art". He also enjoys cooking, wine tasting and traveling.

**Gerard McLauchlan** is a European and Royal College Recognised Specialist in Internal Medicine. He was a Senior Lecturer at the University of Glasgow before completing a 12 month training period in Interventional Radiology at the Animal Medical Center in New York. In April 2017 he joined Fitzpatrick Referrals as a Senior Clinician in Interventional Radiology where he works in the dedicated Soft Tissue and Oncology Hospital.

**Prof. dr. Björn Meij**

**Department Clinical Sciences of Companion Animals**
**Faculty of Veterinary Medicine**
**Utrecht University, The Netherlands (NL)**

Current position: Professor of Small Animal Surgery, Utrecht, NL

**Education & Career:**
- Veterinary Medicine (1980-1986, University of Ghent, Belgium)
- Internship and residency Small Animal Surgery (1986-1991, Utrecht, NL)
- RNVA Veterinary Specialization, Small Animal Surgery, (1991, Utrecht, NL)
- European College of Veterinary Surgeons (1993, Cambridge, UK)
- PhD cum laude (Utrecht University, NL, 1997).
- Fellowship Neurosurgery (1999-2000, School of Medicine, University of Virginia, Charlottesville, USA)
- 2008, Utrecht, NL: Associate Professor, Orthopedics & Neurosurgery
- 2014, Utrecht, NL: Professor and Head of Small Animal Surgery

**PhD Thesis:** Transsphenoidal hypophysectomy for treatment of pituitary–dependent hyperadrenocorticism in dogs. 30.10.1997, Utrecht. Cum laude.

**Specialization: Orthopedics and Neurosurgery**

**Research focus:**
1. Intervertebral disc degeneration in the dog, spinal surgery, cranial surgery.
2. Oncogenesis and surgery of pituitary adenomas in the dog and cat.

**Dr. Søvik** is both a veterinarian and a medical physicist. She graduated from the University of Oslo with an MSc in Biophysics in 2002 and a PhD in Medical physics in 2007. Her doctoral thesis and subsequent postdoctoral research were performed at the Oslo University Hospital, focusing on biologically adapted, image guided radiation therapy. Concurrently, she studied veterinary medicine at the Norwegian School of Veterinary Science and obtained her DVM in 2008. She went on to complete a rotating small and large animal internship, as well as a diagnostic imaging residency at the Norwegian School of Veterinary Science and became a Diplomate of the European College of Veterinary Diagnostic Imaging in 2013. Currently, she is an Associate Professor in Veterinary Diagnostic Imaging at the Norwegian University of Life Sciences. Her current research interests include the use of artificial intelligence for image analysis in veterinary oncology and veterinary radiation protection.

**Dr. Tselikas** is an interventional radiologist working in Gustave Roussy, the largest European cancer institute. He is specialized in cancer treatment interventions, and has special interests in tumors percutaneous ablation techniques including lung, liver, kidney and bone tumor local treatments.
He also specializes in intra-arterial procedures, specifically for the treatment of liver primary and secondary tumors.
He has participated and was very implicated large prospective international trials on lung and bone cryoablation, and intra-arterial therapies such as radioembolization and chemoembolization.
Dr. Tselikas current research topics are the combination of interventional radiology technics with immuno-oncology and local administration of immunotherapies in cancer patients.
He is member of the editorial board of CVIR and Diagnostic and Interventional Imaging, and imaging and interventional radiology societies such as SFR, CIRSE and SIO.

**Dr. Vail** is Professor and Barbara A. Suran Chair in Comparative Oncology at the University of Wisconsin-Madison and the UW Carbone Comprehensive Cancer Center. He received his DVM from the University of Saskatchewan in 1984 and a residency in Medical Oncology at the Animal Cancer Center at Colorado State University in 1990. Dr. Vail has published over 150 peer-reviewed scientific manuscripts, 50 book chapters, and is co-editor of the textbook Small Animal Clinical Oncology. He is past president of the Veterinary Cancer Society and recipient of both the Mark L. Morris Sr. and Pfizer Award for Veterinary Research Excellence.

**Maïa Vanel** graduated from the Vet School of Nantes in France. She then performed an internship at the University of Montreal and her residency in France and Belgium. She worked several years in a veterinary hospital in Nantes and is now working in AniCura TRIOVet in Rennes, with a special interest in oncology.

**Prof Daniel Vanel** Radiologist. Former chairman of Radiology, the Gustave Roussy Institute, Villejuif, France
Former chief of research and teaching imaging of musculo skeletal tumors, The Rizzoli Institute, Bologna, Italy. Ex-president of the European Skeletal Society of Radioloy, and International Cancer Imaging Society. Gold medallist of the International Skeletal Society.

# ESVONC Congress 2021

## Virtual Congress, 28-29 May 2021

# THEMED SESSION - Rescue Protocols: What to do when first line fails

# Lymphoma Rescue with Cytotoxic Chemotherapy – A Cynics Perspective

David M. Vail DVM, DACVIM(Oncology)
Professor and Barbara A. Suran Chair of Comparative Oncology
School of Veterinary Medicine and the Carbone Cancer Center,
University of Wisconsin, Madison, Wisconsin, USA (david.vail@wisc.edu)

Eventually, the vast majority of dogs and cats with intermediate or high-grade lymphoma that achieve a remission will relapse. This usually represents the emergence or section of tumor clones or tumor stem cells that are inherently more resistant to chemotherapy than the original tumor, the so-called MDR clones that either were initially drug resistant or became so following exposure to selected chemotherapy agents. Evidence suggests that in dogs with relapsed lymphoma, tumor cells are more likely to express genes that encode ABC-transporter protein transmembrane drug pumps often associated with MDR. MDR1 represents only one of the plethora of mechanisms that lead to drug-resistant disease. Other causes for relapse following chemotherapy include alteration in molecular death-pathways (e.g., pro-apoptosis pathways), chemoresistant tumor stem cells, inadequate dosing and/or frequency of administration of chemotherapy, and failure to achieve high concentrations of chemotherapeutic drugs in certain sites such as the CNS.

The overriding goal of relapse or salvage therapy in veterinary oncology is quite different than that for physician-based oncology. In people at relapse, the overriding goal is to achieve a short-term reinduction in preparation for high-dose chemotherapy followed by autologous/allogeneic stem cell transplant. In contrast, we (veterinarians) do not generally have globally accessible nor feasible transplant options; therefore, we are asking a great deal from cytotoxic chemotherapy-based rescue protocols. It is likely, therefore, no matter how complicated or aggressive our chemotherapy-based rescue protocols become, durability will always be a significant hurdle in veterinary oncology – and this is borne out by currently available outcomes between single-agent and complex rescue protocols.

At the time of first relapse, it is recommended that reinduction be attempted first by reintroducing the induction protocol that was initially successful, provided the recurrence occurred temporally far enough from the conclusion of the initial protocol (e.g., ≥ 4 months) as reinduction success is likely in those scenarios. The cumulative dose of doxorubicin (DOX), baseline cardiac assessment, the use of cardioprotectants, alternative drug choices, and client education should all be considered. In general, the duration of reinduction will be half that encountered in the initial therapy; however, a subset of animals will enjoy long-term reinductions, especially if the dog completed the initial induction treatment protocol and was currently not receiving chemotherapy for several months when relapse occurred. Reinduction rates of nearly 80% to 90% can be expected in dogs that have completed CHOP-based protocols and then relapse while not receiving therapy. The duration of a second CHOP-based remission in one report was predicted by the duration of the interval between protocols and the duration of the first remission.

If reinduction fails or the dog does not respond to the initial induction, the use of so-called “rescue” or “salvage” agents or protocols may be attempted. These are single drugs or drug combinations that are typically not found in standard CHOP protocols and are withheld for use in the drug-resistant setting. The most common rescue protocols used in dogs include single-agent use or a combination of (in alphabetical order not order of superior efficacy) actinomycin D, bleomycin, dacarbazine (DTIC), DOX (if not part of the original induction protocol), etoposide, L-asparaginase, lomustine (CCNU), mechlorethamine, melphalan, mitoxantrone, prednisone, procarbazine, rabacfosadine, temozolomide, vinblastine, and vinorelbine. Some rescue protocols are relatively simple and convenient single-agent treatments, whereas others are more complicated (and expensive) multiagent protocols, such as MOPP (mechlorethamine, vincristine, procarbazine, prednisone). Overall rescue response rates of 40% to 90% are reported; however, responses are usually not durable with median response durations of 1.5 to 2.5 months being typical regardless of the complexity of the protocol. Please see Table 33.5 of the 6th edition of Small Animal Clinical Oncology and the other references following this proceeding for an exhaustive list of currently published rescue protocols in dogs and cats. One report of MOPP in relapsed cats with lymphoma resulted in more durable remission durations; however, cats were not categorized into low vs intermediate or high-grade lymphomas and indeed the majority (>60%) of cases were reported as gastrointestinal, likely representing low-grade lymphomas better managed with less aggressive rescue protocols. Further, the vast majority (>80%) were at first-relapse with no reinduction attempted, a scenario often associated with more durable remissions. Finally, another MOPP report in cats with intermediate/large cell lymphoma in a more typical rescue setting had median response durations of only 39 days. A small (< 20%) subset of dogs and cats will enjoy longer rescue durations. Current published data from rescue protocols do not include sufficient numbers for adequate statistical power, nor do they compare protocols in a controlled, randomized prospective fashion. Therefore, comparative evaluations of efficacy among various protocols are subject to substantial bias, making direct comparisons difficult. Choice of a particular rescue protocol should depend on several factors, including cost, time commitment required, efficacy, adverse event profile, and experience of the clinician with the protocols in question.

As the complexity of rescue protocols does not yet appear to be associated with significant gains in rescue durability, the author tends to choose simpler and less costly protocols (e.g., CCNU/L-asparaginase/prednisone); however, the use of multiple varied rescue protocols, switching as needed based on response, continues as long as clients are comfortable with their companions quality of life. This sequential application of several different rescue protocols can result in several months of extended survival with acceptable quality of life.

**Strategies to Enhance Effectiveness of Cytotoxic Therapy in Lymphoma**

Despite the plethora of published chemotherapeutic rescue protocols for dogs and cats with lymphoma, it appears we have achieved as much as we can from currently available cytotoxic chemotherapeutics in standard settings. The 1.5-2.5 month median response “wall” has not improved dramatically. Further advances in remission and survival durations await the development of new methods of delivering or targeting traditional chemotherapeutic drugs, new generations of chemotherapeutic drugs, or novel non-cytotoxic chemotherapeutic treatment modalities (alone or in combination with cytotoxic chemotherapy protocols). In particular, the development of

immunotherapies, targeted small molecule therapy, novel radiation therapy, epigenetic modification and more feasible/accessible transplant technologies. These are all active areas of investigation in both human and veterinary medicine and will be discussed in the following session.

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# Beyond Cytotoxic Chemotherapy – Novel Approaches to Lymphoma Rescue

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In physician-based oncology, many of the novel approaches discussed in this lecture may actually be applied in an up-front induction setting prior to relapse based on known prognostic/predictive characterizations at initial diagnosis. For example, certain genetic, chromosomal, or signalling pathway sub-classifications may warrant initial aggressive approaches such as stem-cell transplant, chimeric antigen receptor T cell (CAR T) cell therapy or other novel approaches. This presentation will discuss several novel approaches that are being evaluated in human lymphoma patients with a focus on those either currently being investigated in veterinary species or that have significant potential in veterinary species. A discussion of Stem or bone marrow transplant will not be a focus of this presentation. As with many cancer treatment modalities, the approaches discussed below will likely have to be applied in combination protocols to ultimately realize durable responses in the majority of patients.

**Small Molecule Inhibitors of Cell Signaling Pathways**
A number of new drugs have recently shown activity against lymphoma, including some that have license in veterinary patients (e.g., Laverdia-CA1). Several B and T cell signaling pathways have been targeted in human therapy trials and several have been investigated in dogs with lymphoma. These include inhibitors of bruton tyrosine kinase (BTK), IL2-inducable T cell kinase (ITK), phosphatidylinositol 3-kinase (PI3K), JAK/STAT, nuclear export, $NF_KB$, MAPK and mTOR, among others. All of the aforementioned pathways have been implicated as drivers of canine lymphoma *in vitro* and inhibition of several of these pathways in phase I/II comparative trials in dogs have shown efficacy in single-agent applications as will be discussed. Ultimately, their inclusion in combination protocols will show the most promise with respect to increasing overall response rates and durability of response.

**Epigenetic Modulation Therapy**
By definition, epigenetic modifications to the DNA of cancer cells do not involve a change in the nucleotide sequence, rather they involve changes in the way the genetic code is expressed. This generally involves 3 potential mechanisms: 1) DNA methylation; 2) histone modifications; 3) regulatory RNA (e.g., microRNA gene silencing).

DNA methylation status determines the folding characteristic of chromatin which ultimately determine the expression of genes and can lead to silencing of tumor suppressor genes which is a hallmark of cancer. Cancer cells tend to have hypermethylated CpG islands preceding tumor suppressor gene promoters which silence expression. Therefore, hypomethylation agents have been developed (e.g., azacytidine, decitabine, guadecitabine) that act through the reactivation of tumor

suppressor genes, repression of oncogenes, restoration of DNA repair mechanisms, anti-angiogenesis properties and activation of immune response pathways. Many are being investigated in human trials of relapsed or refractory lymphoma. Aberrant methylation status has been documented in a canine NHL samples and feline NHL cell lines and azacytidine therapy has shown activity in dogs with invasive urothelial carcinoma suggesting hypermethylation is a clinically relevant target for further exploration in veterinary species.

Histones are proteins involved in folding and compaction of chromatin which also effects gene expression. Histone modifications, particularly methylation and acetylation, are generally involved in chromatin state regulation and tumor progression. Histone deacetylase inhibitors (HDACi) such as romidepsin and chidamide are actively being investigated in human trials of relapsed or refractory lymphoma, either alone or in combination with chemotherapy. Further, canine NHL cell lines are sensitive to HDACi and a phase I trial of the HDACi valproic acid has been performed that included dogs with NHL.

Targeting regulatory RNA is an emerging field of cancer therapy. MicroRNA (miRNA) are small noncoding RNA sequences which can post-transcriptionally regulate protein synthesis. It is estimated they regulate 60% of the transcriptional activity of protein-encoding genes. In human B and T cell malignancies, specific miRNAs are overexpressed and can serve as both diagnostic biomarkers and therapeutic targets through the use of designer miRNA inhibitors. Tumor specific miRNAs have been characterized from dogs with lymphoma suggesting they may also serve a similar diagnostic and therapeutic role in the species.

**Immunotherapeutic Approaches**

Monoclonal Antibody (MAb) Approaches

Enhanced durability of first remissions in humans with B cell NHL has been achieved primarily through the inclusion of monoclonal antibody (MAb)-based therapies (e.g., anti-CD20, rituxamab) to standard CHOP protocols. Recently, caninized MAb designed to target either B-cell (blontuvetmab) or T cell (tamtuvetmab) lymphoma in dogs were conditionally approved by the USDA; however, target specificity was found to be inadequate to effect clinical efficacy. Several laboratories are working to develop more specific and effective MAb therapies for use in canine lymphoma.

Anti-checkpoint MAbs (e.g., anti-PD-1, CD47) are another approach being investigated to treat relapsed/refractory lymphoma in people. Anti-PD-1 therapy has also been used with some success as a rescue agent in people with certain B and T cell lymphomas. A potential drawback with this approach is that when used to treat T cell lymphomas, the malignant T cells may express the same receptors as the intratumoral cytotoxic T cells that potentially could target the malignancy. This theoretically could results in 'hyper-progression' of T cell lymphoid cancers – a phenomena that has been documented in a subset of people treated with anti-PD-1 therapy and recently resulted in the stoppage of a clinical trial involving Nivolumab (humanized anti-PD-1) rescue in people with peripheral T cell lymphoma. In contrast to anti-PD-1 therapy, CD47 serves as a "don't eat me signal" primarily involving the innate immune system; activated macrophages that encounter CD47 on tumor cells will not phagocytise them. CD47 expression has been documented to be elevated in some forms of lymphoma in people. Thus, anti-CD47 MAbs could enable macrophage phagocytosis of cancer, and

therefore may be less likely to result in hyper-progression when used against T cell lymphomas. Humanized anti-CD47 MAbs are currently in clinical trials in people with diffuse large B cell lymphoma (DLBCL), follicular lymphoma and various T cell lymphomas. It appears to be particular effective when MAbs against relevant tumor associated antibodies are used concurrently, thereby providing an antibody Fc region for antibody dependent cellular cytotoxicity (ADCC) co-signalling. In a similar way, CD47 blockade in a xenograft model of canine DLBCL was shown to synergise activity with CD20 blockade.

Anti-CD30 MAbs (e.g., brentuximab) represent another approach being investigated in human lymphoma. CD30 (TNFRSF8) is a receptor expressed on activated (but not resting) T and B lymphocytes and neoplastic mast cells in people. Brentuximab is currently FDA approved in the rescue setting for Hodgkin's lymphoma, anaplastic large cell lymphoma, primary cutaneous anaplastic large cell lymphoma and CD30-expressing mycosis fungoides in people. Clinical trials of brentuximab are also ongoing in people with various types of CD30-expressing B and T cell lymphoma and NK cell lymphoma. Expression of CD30 has been documented in canine anaplastic large T cell lymphoma and neoplastic mast cells, and some B and T cell lymphomas in cats. As such, CD30 could serve as a target for therapy in these species. Additionally, anti-CD30 CAR T cells will be discussed in a section dealing with CAR T cell therapy below.

Anti-checkpoint MAbs (e.g., anti-PD-1, CD47) are another approach being investigated to treat relapsed/refractory lymphoma in people. Anti-PD-1 therapy has also been used with some success as a rescue agent in people with certain B and T cell lymphomas. A potential drawback with this approach is that when used to treat T cell lymphomas, the malignant T cells may express the same receptors as the intratumoral cytotoxic T cells that potentially could target the malignancy. This theoretically could results in 'hyper-progression' of T cell lymphoid cancers – a phenomena that has been documented in a subset of people treated with anti-PD-1 therapy and recently resulted in the stoppage of a clinical trial involving Nivolumab (humanized anti-PD-1) rescue in people with peripheral T cell lymphoma (5). In contrast to anti-PD-1 therapy, CD47 serves as a "don't eat me signal" primarily involving the innate immune system; activated macrophages that encounter CD47 on tumor cells will not phagocytise them. CD47 expression has been documented to be elevated in some forms of lymphoma in people. Thus, anti-CD47 MAbs could enable macrophage phagocytosis of cancer, and therefore may be less likely to result in hyper-progression when used against T cell lymphomas. Humanized anti-CD47 MAbs are currently in clinical trials in people with diffuse large B cell lymphoma (DLBCL), follicular lymphoma and various T cell lymphomas. It appears to be particular effective when MAbs against relevant tumor associated antibodies are used concurrently, thereby providing an antibody Fc region for antibody dependent cellular cytotoxicity (ADCC) co-signalling. In a similar way, CD47 blockade in a xenograft model of canine DLBCL was shown to synergise activity with CD20 blockade.

Finally, MAbs targeting chemokine receptors have shown some promise for treating relapsed T cell lymphoma in people. Mogamulizumab, a MAb which targets CC chemokine receptor 4 (CCR4) is FDA approved for treating relapsed or refractory CCR4+ adult T cell leukemia/lymphoma and cutaneous T cell lymphoma in people. CCR4+ lymphocytes exist in dogs and CCR4 blockade (with mogamulizumab) depleted regulatory T cells and prolongs survival in canine bladder cancer and another

CCR4 antagonist was effective in treating canine atopy implying this is a druggable target in the species.

Of course, any of these humanized MAb therapies are not likely to be useful long-term in dogs until caninized versions are developed.

Adoptive Immunotherapy Approaches

Much excitement has been generated in physician-based medicine about adaptive immunotherapy approaches, in particular the application of CAR T therapy. CAR T are T cells that have been genetically engineered to produce an artificial T cell receptor. The role of CAR T cell therapy in relapsed/refractory DLBCL in people is well established. CD19-targeting CAR T cell therapy has been FDA approved for relapsed/refractory acute lymphoblastic leukemia and DLBCL in people. Nicola Mason's group has manufactured CD20-targeting CAR T cells using a lentivector in dogs with DLBCL and shown some degree of activity; albeit many of the challenges associated with human CAR T therapy are present. With respect to T cell lymphoma, CAR T therapy comes with challenges not seen when targeting B cells – in particular, CAR T therapy may result in fratricide (i.e., CAR T killing CAR T) leading to an inability to manufacture cells, a loss of efficacy in vivo and ablation of normal T lymphocytes leading to profound immunosuppression. Targeting T cell antigens restricted to malignant T cells may overcome these problems. One potential target is CD30 and CD30-targeting CAR T cells are showing preliminary activity in people with T cell malignancies.

Autologous T cell infusions are also currently in clinical trials in dogs with various tumors, including B cell lymphoma. These trials are currently limited to B cell lymphoma owing to concerns previously discussed on the risks of hyper-progression using T cell stimulating therapy in patients with T cell lymphoma. A survival advantage has been suggested when autologous T cells were infused following CHOP chemotherapy in a small number of dogs with DLBCL when compared to stage-matched historical controls.

Antitumor Vaccine Approaches

Several antitumor vaccine approaches have been investigated in dogs with lymphoma, including tumor vaccine extract using killed lymphoma cells combined with Freund's adjuvant and autologous killed and/or gene engineered lymphoma tumor cell vaccines; however, no significant gains in remission times or overall survival have been documented. Exploratory vaccines targeting telomerase, heat shock proteins, and RNA-loaded CD40-activated B cells in dogs with lymphoma have also been conducted. These studies involved small numbers of nonrandomized patients and lacked controlled populations for comparison. A xenogeneic DNA vaccine designed to target canine CD20 is currently undergoing clinical trials in the USA. Although preliminary activity is suggested in many of these reports and they are serving to enhance our basic understanding of immunotherapeutic methodologies, their development is still early; complete safety and efficacy trials have not been completed to date.

**Radiation Therapy Approaches**

There is some familiarity in the veterinary literature with the use of traditional external beam radiation therapy (EBRT) for regional rescue therapy in dogs and cats and for whole body or consecutive half-body EBRT consolidation therapy. However, the use of EBRT is currently limited to those utilities unless some type of Stem or BM transplant rescue can follow.

The area showing more promise for treating systemic or multicentric lymphoma in people is the use of molecularly targeted radionuclide therapy (TRT). TRT can selectively deliver lethal radiation doses to tumor cells while sparing normal tissues. This approach is effective in treating humans with relapsed/refractory B cell lymphomas using FDA approved radiolabeled anti-CD20 antibodies ($^{131}$I-tositumomab; $^{90}$Y-ibritumomab). Currently, adequate T cell targets for a TRT approach do not exist for people and neither B or T cell targets exist for use in veterinary species. An alkylphospholipid analog (NM600) that chelates radionuclides has shown significant promise as a TRT agent in murine lymphoma models and we have been investigating its use as a theranostic agent in several tumor histologies in companion dogs using the $^{86}$Y/$^{90}$Y isotope pair. We are currently investigating its use as a theranostic in dogs with T cell lymphoma, using $^{86}$Y-NM600 to image and create dosimetry for $^{90}$Y-NM600 therapeutic intervention – some preliminary proof of concept in companion dogs will be presented.

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# Interventional Oncology – Embolization and Microwave Ablation

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Interventional oncology (IO) is regarded as the 4th pillar of human oncology alongside medical, surgical and radiation oncology. Interventional oncology can either be used as a primary treatment, to palliate clinical signs or in an attempt to shrink a tumour and make surgical removal possible. In veterinary medicine IO primarily involves trans arterial therapy (intra-arterial administration of a chemotherapeutic drug or embolic agent) or ablative therapy (thermal such as microwave ablation or chemical such as ethanol ablation.

Intra-arterial (IA) chemotherapy normally involves vascular access being obtained via the femoral or carotid artery. Using fluoroscopic guidance, the clinician then selectively delivers the cytotoxic agent directly to the tumour via its arterial supply. The IA administration of chemotherapy agents is not associated with an increased level of drug-associated side effects and in one study systemic side effects occurred significantly less commonly following IA administration compared to IV administration (Culp at al 2015). The effect of IA chemotherapy on disease free interval and survival compared to IV administration remains contentious.

Embolization involves the delivery of particles to the feeding arterial supply of tumours (+/- concurrent chemotherapy – known as chemoembolization). This technique not only increases local concentration of chemotherapeutic agents but the embolisation itself results in tumour cell necrosis. Advanced multiphase cross-sectional imaging is essential for embolisation procedures and the techniques should only be attempted by those with advanced training and intricate knowledge of the relevant vascular anatomy to avoid complications including non-target embolisation.

In veterinary patients most research and clinical work has been focused on non-resectable hepatocellular carcinomas and lower urinary tract neoplasms.

## Non-resectable HCC

Solitary hepatocellular carcinomas (HCC) should be regarded as a surgical disease in dogs (median survival time >3y following complete excision). In patients in whom the neoplasm is considered non-resectable or is diffusely infiltrating the liver or in which owners decline surgery there is the option of transarterial chemoembolization (TACE) or bland embolization. The evidence for performing a TACE over bland embolization is limited. As HCC receive almost their entire blood supply from the hepatic artery whereas the normal liver receives most of its supply from the portal vein it makes them particularly susceptible to TACE / bland embolization (Goode 2019). Access is gained via the femoral artery and using fluoroscopic guidance this is followed by selection of the celiac and common hepatic artery. After this superselection of the specific arterial branches supplying the tumour is achieved to ensure minimal non-target embolization occurs (care in particular must be taken to avoid embolization of the gastroduodenal artery as this may result in significant

morbidity, in particular pancreatitis). The combined chemotherapy and embolization agent (most commonly polyvinyl alcohol particles) is administered into the feeding arteries and a repeat contrast fluoroscopy study performed to confirm vascular stasis within the tumour. Following chemoembolization a HCC does not generally significantly reduce in volume (10-30%) however repeat CT angiogram 6-8 weeks post embolization will often show marked changes in the attenuation of the mass indicating significant tumour necrosis. The aim should be “stable disease” and owners should be made aware that the procedure may need to be repeated every 3-6 months to limit tumour growth.

Lower urinary tract tumours

With regards urinary tract tumours there are various studies that have shown higher local chemotherapy concentrations and improved remission rates in laboratory animals that received IA chemotherapy versus traditional intravenous administration (Hoshi et al 2007). A study in research beagles documented that is was possible to achieve >8x increase in bladder, prostate and regional lymph node chemotherapy concentration following IA administration compared to dogs receiving the drug via the traditional intravenous route (Sumiyoshi et al 1991). IA chemotherapy has been shown to result in increased tumour drug concentration and may therefore result in increased efficacy. In dogs with naturally occurring prostate carcinoma the use of IA carboplatin was shown to result in significantly more patients entering clinical remission (as judged by the RECIST criteria) and suffering from chemotherapy associated side effects (anaemia, lethargy and anorexia) compared to those receiving iv carboplatin (Culp et al 2016). The author has commonly been treating bladder, urethral and prostatic neoplasia with intra-arterial chemotherapy alongside other modalities including surgery and intravenous chemotherapy with a positive response. Embolisation of the prostatic artery has recently been presented (non published – Culp) and shows promising results. Following targeted intra-arterial therapy (either chemotherapy or embolisation) systemic chemotherapy should be administered to treat metastatic disease. The author has now embolised in excess of 40 prostatic tumours with excellent results seen in many cases.

# Interventional radiology for oligometastatic disease and cancer palliation

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Interventional radiology, and more specifically, interventional oncology (IO) plays a key and increasing role for the management of oligometastatic disease and cancer palliation.

Percutaneous ablation technics, such as Radiofrequency ablation (RFA), Microwave ablation (MWA) and more recently cryoablation and irreversible electroporation allow for minimal invasive tumor destruction. For selected patients, with either synchronous oligo*metastatic* (the definition can vary according to the primary tumor), oligo*progressive*, or oligo*resistant disease* this approach can improve both progression free and overall survival.
These technics can be applied to various organs such as liver, kidney, lung, or even bone tumors. Tumor sampling can be performed during the same procedure.
Because of the very low risk of complication, these approaches are becoming more and more popular for metastatic patients.

Loco-regional treatments mainly represented by intra-arterial therapies (bland embolization, chemoembolization and radioembolization) can also be used for liver dominant or liver only metastatic disease, with very high objective response rates among various diseases.

Finally, palliation becomes of major importance in the global management of cancer. IO allows for effective pain palliation using both ablation and consolidation technics. Indeed, bone tumor involvement, is frequently responsible for pain and quality of life impairment. Cementoplasty and percutaneous osteosynthesis are very effective on mechanical disorders caused by cancer, and combination with percutaneous ablation, neurolysis and pain killers administered locally result to a nearly complete alleviation of pain in a large number of patients.

Other palliative procedures include drainages or urgent embolization in order to overcome critical situations.

# THEMED SESSION - How Advanced Imaging is and will be changing Oncology

# Advanced/Functional Imaging (PET/CT, PET/MR) in Companion Animal Cancer

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Advanced molecular imaging techniques, which measure biologic processes at the cellular level are quickly becoming commonplace in physician-based oncology and have the potential to play an important role in the tailoring of cancer therapy in veterinary patients. Advanced imaging modalities have several applications, including the early detection of cancer, more accurate staging of cancer, determination of response to therapy (including prediction of response) and early detection of cancer recurrence. Further, advanced imaging modalities that provide information on the functional properties of tumors, including metabolic rate, proliferative status, vascularity/perfusion, presence of receptors/tumor associated antigens, and assessment of the tumor microenvironment (TME) are now within our grasp. Importantly, functional imaging modalities open up the possibility of developing and enhancing so called “theranostic” approaches whereby specific imaging tracers and modalities can simultaneously or near-simultaneously diagnose and treat the cancer in question. Additionally, with the development and availability of advanced image-modulated radiation therapy delivery devices (e.g., Radixact™, Trilogy™, Tomotherapy™), the co-development of advanced imaging technologies are critical in order to take advantage of these advancements. While the benefits of more accurate and extensive imaging modalities are intuitive, there exist inherent problems created by their addition into the current standard of care. That is, if we are able to diagnose tumors earlier, 'lead time bias' – the increased survival that results from early detection - can confuse comparisons of treatment and population groups from that point forward. Additionally, the ability to stage tumors more thoroughly may place patients in a more negative prognostic categories when indeed 'stage-migration' has made this artificial. As most of us are familiar with standard advanced imaging modalities such as magnetic resonance (MR), computed tomographic (CT), and positron emission tomography (PET) imaging, this discussion will be limited to newly developed enhancements and applications of these modalities.

## The Concept of RADIOMICS

A rapidly expanding field of medical image analysis, so called “RADIOMICS”, is harnessing the full power of medical imaging by extracting numerous quantitative features (sometimes referred to as “texture features”) out of the images of different modalities, including PET, CT and magnetic resonance imaging (MRI). RADIOMICS analysis provides a wealth of additional previously hidden information about tumors that is not visible to the naked eye, including quantitative information about the spatial heterogeneity of the imaged tumors, which may be further correlated with heterogeneity of response to different oncologic therapies, tumor aggressiveness, progression-free and overall survival. Through the use of advanced data management, RADIOMICS can even be used to predict risk of cancer development, histologic phenotype, response to targeted therapy, likelihood of adverse events and likelihood of recurrence.

Indeed, some algorithms can allow advanced imaging to act as a “virtual biopsy” of the entire tumor burden over many serial time-points. RADIOMICs has been applied to canine nasal tumors to show prediction of histologic phenotype and superior staging utility. Further, functional imaging of canine nasal tumors may help plan radiation therapy with respect to where therapeutic ‘boost’ may benefit based on areas of hypoxia or proliferation.

**Assessing and/or Predicting Clinical Response During Cancer Therapy**

The utility of advanced imaging to serially assess functional changes in tumors or TME to document or indeed predict response to cancer therapeutics is current clinical practice in physician-based oncology and is now being both applied and investigated in companion species. For example, the authors validated the non-invasive assessment of tumor proliferation of the thymidine-analogue 3’-deoxy-3’[$^{18}$F]fluorothymidine (FLT) by comparing FLT uptake in companion dogs with non-Hodgkin’s lymphoma (NHL) to gold-standard Ki-67 immunohistochemistry. Comparisons of $^{18}$F-FDG and $^{18}$F-FLT may also allow distinction of tumor borders within areas of higher-than-average background tracer uptake, such as within liver or brain. These studies illustrate the use of advanced imaging to document efficacy of investigational cytotoxic agents like the novel cytotoxic prodrug, rabacfosadine, and also to map areas of chemosensitive cells such as proliferating bone marrow.

**Assessing Clinical Response To Cancer Immunotherapy**

Although immunotherapy is becoming one of the cornerstones of modern cancer therapy resulting in durable favourable outcomes for patients, the assessment of clinical response to immunotherapy is still a very challenging task. Immunotherapy response patterns can be substantially different from those of classical cytotoxic therapies. A significant subset of patients first experience a pseudo-progression after the administration of immunotherapy, and the actual response/shrinkage of tumours can be delayed and only observed later in the time course of therapy. Therefore, the standard Response Evaluation Criteria in Solid Tumours (RECIST 1.1.) are not appropriate for assessing the effects of immunotherapy. Immune-related response criteria (irRC or iRECIST), are now recommended for use in immunotherapy. Broadly speaking, iRECIST only covers assessment of anatomical changes, which are known to be slow, compared to molecular and functional changes within the tumor and tumor microenvironment, and has been inconsistently implemented. Ideally, methods that assess response to immunotherapeutic strategies as early as possible should allow non-responding patients to switch to other treatment modalities sooner and guard from the high cost and toxicities of continuing an ineffective therapy. We are currently evaluating molecular imaging-based RADIOMICS analysis of FDG PET and FLT PET to provide more information than standard anatomical imaging-based analysis regarding the assessment of the effectiveness of immunotherapy, a so called iRADIOMIC approach. A positive immunotherapeutic response should be reflected by an increased FDG/FLT uptake ratio because of two premises: 1) effective immunotherapy elicits immune activation leading to increased “inflammation” in the tumor reflected by increased glycolytic activity in the tumor microenvironment by activated immune cells, which can be measured by FDG PET, and 2) effective immunotherapy will eventually result in antitumor effects, which can be measured by a decrease in “proliferation” as reflected by thymidine synthase activity in the tumor region measured using FLT PET. We hypothesize that the change in the ratio of FDG to FLT will provide the necessary discriminatory information to

characterize tumor lesions as having a *positive immune effect* (increase in FDG/FLT ratio) versus *no effect* (no change or decrease in FDG/FLT ratio).

**Development of Novel Theranostic Approaches**

Much utility is gained in clinical practice if a single agent can be used for both diagnostic imaging and therapy; so called "theranostic" agents. For example, metaiodobenzylguanidine (mIBG) is such an agent for pediatric neuroblastoma where $^{123}$I-mIBG is used for accurate staging and $^{131}$I-mIBG is the correlate therapeutic. Similarly, $^{177}$Lu prostate-specific membrane antigen ($^{177}$Lu-PSMA) is used as a theranostic for men with prostate cancer.

Our group is working with radiolabeled alkylphophocholines (APCs) which selectively accumulate in tumor cells *in vivo* by exploiting the relative overabundance of lipid rafts in cancer versus normal cells, a mechanism that is ubiquitous to most malignancies. An APC analog, NM600, developed by members in our group targets numerous cancer types regardless of histology and anatomic location. NM600 chelates a variety of radiometals (e.g., $^{86}$Y, $^{90}$Y, $^{177}$Lu, $^{225}$Ac) and is currently being evaluated in multiple imaging/therapy trials. Members of our collaborative group have eloquently shown that distant metastatic sites serve as a nidus for immunosuppressive cells (e.g., Tregs), and these mediate systemic immunosuppressive effects that antagonize external beam radiation therapy (EBRT) generated *in situ* vaccination – a phenomenon called concomitant immune tolerance (CIT). Fortunately, CIT is radiation sensitive; delivering low-dose (~ 2 Gy) RT to metastatic sites can overcome CIT and enable *in situ* vaccine regimens to destroy both primary and distant tumor. While it is not typically feasible to deliver EBRT to all sites of metastatic disease (due to immune suppression and inability to specifically target all microscopic disease), it is possible to use targeted radionuclide therapy (TRT) to immunomodulate the TME of all tumor sites in the setting of metastatic disease. We are currently investigating delivery of low dose TRT to all tumor sites in the setting of metastatic disease by using the theranostic isotope pair, $^{86}$Y-NM600 and $^{90}$Y-NM600, to immunomodulate the collective TME in a way that will promote response to EBRT-based *in situ* vaccine. Radiolabeled NM600 enables tumor-specific PET imaging ($^{86}$Y-NM600) and targeted delivery of ionizing radiation ($^{90}$Y-NM600) at doses that theoretically will abrogate CIT. These data proved selective uptake of NM600 by all metastatic sites and allowed dosimetry calculations that predicted at least a 2:1 tumor to bone marrow differential uptake and safe delivery of $^{90}$Y-NM600 to all metastatic tumors at doses likely to overcome CIT while sparing bone marrow.

**Assessment of Cellular Trafficking**

The ability to image, in real time, changes in tumor and TME infiltrating immune effector and suppressor cell trafficking would be highly advantageous to assess effectiveness of immunotherapeutic approaches and characterize cell-based immune approaches. By way of example, we and others have been investigating the utility of natural killer cell (NK) based therapies in companion dogs. Confirming delivery of cell therapies to tumors and other sites of disease will become more important as treatments are tailored to individual patients or modulated over time with repeated dosing. MRI is the clinical standard for obtaining nonradioactive high-resolution images of soft tissue including solid tumors. While conventional MRI detects tissue $^{1}$H, and mainly differences in signal recovery of water and fat, multinuclear and spectroscopic MRI has the potential to detect functional and cellular signals not visible with conventional $^{1}$H MRI methods. $^{19}$F MRI is a promising approach for tracking NK cells noninvasively

without toxicity or ionizing radiation. Members of our collaborative group have developed methodology to label canine NK cells with non-radioactive $^{19}F$ without compromising NK cell function and they were the first group to enumerate and track NK cells within a tumor *in vivo* using hot spot $^{19}F$-MRI. We are currently evaluating the utility of a customized $^{19}F$ MR coil large enough to acquire images from canines. We have collected and expanded canine NK cells from University of Wisconsin Veterinary Care patients and have begun investigations to refine $^{19}F$-MR imaging protocols to characterize trafficking and persistence of autologous canine NK cells after intratumoral and intravenous infusion. Success of this line of investigation would offer a nonradioactive approach of tracking *ex vivo* activated NK cells (and other immune cells) in patients with solid tumors undergoing immune-based therapies.

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# Osteosarcoma. From Imaging to Therapy, and from Therapy to Imaging. Is it different between humans and dogs?

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Osteosarcoma is the less rare primary malignant bone tumor in humans. Survival has moved from 20 to 66%, mainly with chemotherapy and limb salvage from zero to more than 80%. The need of a very precise local evaluation has been the key of a very precise surgery, and this need has pushed the radiologists to improve their image quality. In the same time new imaging capabilities has allowed and pushed the surgeons to more aggressive and precise local treatments. Imaging plays a major role in diagnosis, with practical therapeutic consequences for the different subgroups, local and general staging, monitoring the treatment and detecting recurrences.

Considerations are slightly different in dogs. Osteosarcoma is also the most common primary bone tumor in dogs. It indeed accounts for more than 85% of all malignant bone tumors and about 70% to 75% of osteosarcomas affects the appendicular skeleton. It occurs mainly in large and giant breeds. However, middle-aged to older dogs are mainly affected, which differs from human medicine, where teenagers to young adults are more commonly concerned. Younger dogs (18-24 months) are also affected by osteosarcomas.

Diagnosis in veterinary medicine is often made lately because the swelling has to be large enough to be noticed by the owner, or because of a pathological fracture. Veterinarian oncologists have therefore to deal with supposedly more advanced disease than in humans. The main objective in vet medicine is to improve the survival time and to have a good quality of life, whereas the cure is the keypoint in human medicine.

Limb amputation is the most common surgical treatment in dogs for appendicular skeleton, especially when the dog is uncomfortable, even though, other surgical techniques exist like limb-sparing surgery. Unlike in humans, and especially in young adults or teenagers, amputation is not a social concern for the dog. Surgical treatment is usually combined to chemotherapy since amputation alone is of course not curative. Stereotactic radiotherapy is a treatment option in dogs which are poor candidates for surgery, or for axial neoplasia, associated or not with chemotherapy. These dogs are however at risk for pathological fractures.

Overall all these diagnostic tools and treatments possibilities, there is a financial concern that does not exist in human medicine whereas it might change the options for the dog, depending on the insurance and the financial possibilities of the owner.

## Imaging diagnosis

Diagnosis of a bone tumor must be teamwork. Combining imaging and histology improves a lot the accuracy and histology must not support the final diagnosis by itself. Radiographs remain the mandatory first step, in human and in veterinary medicine. Classical central osteosarcoma is often obvious: aggressive lesion of the knee, often

calcified, destroying the cortex, invading the soft tissues with periosteal reaction. If the lesion on radiographs is probably malignant, the next step should be immediate MR to stage the lesion.

Radiographs have limitations: superimpositions, partial cortex destruction could be overlooked, flat and short bones and soft tissues are poorly analyzed.

In veterinary medicine, the front limbs are most often affected, especially the distal radius and the proximal humerus. The stifle is also a preferred location on the hindlimbs. Radiographically, at time of diagnosis, an aggressive metaphyseal monostotic lesion is indicative of a primary bone tumor.

*Local staging*
CT is used in case of a diagnostic problem on radiographs. It allows a better study of the cortex, to detect and analyze small calcifications and thin periosteal bone formations. In veterinary medicine, however, CT is more and more widely used, for local staging but also for tumor staging. Indeed, intramedullary and endosteal CT abnormalities are described as accurate to evaluate the length of the tumor. CT can also be used as a prognostic factor as extension to the subchondral bone on CT is also associated with a shorter time to fracture when the dog undergoes a stereotactic radiotherapy treatment.

In humans, the contrast remains however much lower than on MRI, which is the main modality for local staging. Its diagnostic role is limited, as calcifications and periosteal bone formations are more difficult to analyze (a black signal on MRI may be a calcification, but also fibrous tissue or chronic bleeding with hemosiderin). Fluid-fluid levels are better depicted than on CT, because of higher contrast and longer examination time.

On MRI, the precise location of the tumor is well analyzed. Intramedullary extension (and the level of surgical resection), skip metastases, soft tissue involvement, and extension to vessels and nerves are easily and reliably detected. The main limitation is articular extension, which could change the surgical technique: if the tumor abuts on the cartilage, joint involvement cannot be reliably predicted. In case of contra-indications (pace makers, and metallic ocular foreign bodies), CT is used, but has a lower accuracy.

MRI is less used in veterinary medicine. It has been compared to CT to evaluate the tumor extent prior to limb sparing surgery, and one study makes it the more accurate for local staging.

*Distant spread*
In humans, bone metastases and multiple lesions are detected on scintigraphy or PET. Total body MRI is more sensitive without irradiation. Pulmonary metastases are seeked by chest CT. Its sensitivity is good, but specificity is lower.

Canine osteosarcoma tends to metastasize to the lungs and the bones, and less often to the lymph nodes and the abdomen. A whole body CT-exam can be used to detect metastasis and also concurrent lesions, since older dogs are more commonly affected.

Bone scintigraphy remains recommended in veterinary medicine for detection of bone metastasis.

*Osteosarcoma subgroups*

Imaging has a key role in diagnosing osteosarcoma subgroups. It is extremely helpful, as low-grade osteosarcomas do not required chemotherapy. Surface osteosarcomas (periosteal, parosteal, high grade surface) descriptions are scarce in veterinary medicine. This could be explained by a lower incidence or by a misdiagnosis, possibly both in radiology and in histopathology.

Rizzoli figures. In blue are the low grade tumors. Precise diagnosis always requires radio-histological correlation.

| | | |
|---|---|---|
| Classical | 2201 | 76.02% |
| Telangiectatic | 188 | 6.05 |
| Parosteal | 140 | 4.08 |
| Secondary | 108 | 3.70 |
| Central low grade | 74 | 2.60 |
| Periosteal | 40 | 1.40 |
| High grade surface | 43 | 1.50 |
| Parosteal dedif | 30 | 1.00 |
| Facial | 25 | 0.90 |
| Small cell | 24 | 0.80 |
| Multicentric | 15 | 0.50 |
| Intra cortical | 2 | 0.10 |

*Evaluation of treatment effectiveness*

High grade osteosarcomas are treated with preoperative chemotherapy. Decrease of lesion size, ossification, and decrease of early contrast medium uptake on scintigraphy and most of all dynamic MRI are signs of an efficient treatment. They become reliable only late after the beginning of chemotherapy. The initial results of PET are not (yet?) much better.

Preoperative chemotherapy is used before limb-sparing surgery in dogs to induce tumor necrosis. On a long term perspective, depending on the first exam and the financial possibilities of the owner, three-views radiographs, abdominal ultrasonography, whole-body CT or bone scintigraphy can be used to follow the extent of the disease in canine osteosarcomas.

*Detection of local recurrences*

In case of suspicion, a local MR can be performed if the prothesis is non-paramagnetic (that is in titanium).

## Summary

Radiographs remain the first step to image an osteosarcoma in humans and dogs. In case of diagnostic problems, the next step is CT. MR is the main imaging modality for local staging, treatment evaluation and detection of recurrences. PET is still under evaluation. In canine osteosarcomas, CT is for now widely used for local and distant staging, in combination with bone scintigraphy. MRI can be used for local staging before a limb-sparing surgery. Histopathology should always be combined with imaging findings, and discussed with the radiologist.

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# Advanced CT and MRI in Veterinary Oncology: Present & Future

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Diagnostic imaging often plays an essential role in the work-up and management of dogs and cats. While radiography and ultrasonography will remain mainstays in veterinary medicine due to availability, cost and ease of use, advanced diagnostic imaging techniques such as CT, MRI and more recently PET will continue to become more utilized in veterinary medicine due to their increased diagnostic capability and increasing availability. This presentation will review current and emerging advanced applications of CT and MRI in the veterinary cancer patient.

**CT angiographic techniques**
With the increasing availability of multidetector CT scanners, high-quality multiphase CT angiography can be easily performed and can improve characterization of pathology in the cancer patient. Although multiphase CT angiography found some possible differences in enhancement patterns between histopathologic types in various organs (e.g., liver, spleen, adrenals), there is some overlap between various lesions, and one cannot rely solely on these characteristics to make a confident diagnosis based on imaging alone (Kutara et at 2017, Yoshida et al 2016, Kutara et 2014, Leela-Arporn et al 2019). However, CT angiography has many uses in the cancer patient, including assessment for vascular invasion in patients with various tumors, e.g., adrenal, cranial mediastinal, or thyroid tumors; assessment of lesion vascularity for diagnostic purposes, e.g., pancreatic triple-phase angiography for insulinoma; assessment of lesion vascularity for therapeutic purposes, e.g., planning embolization of unresectable liver masses or arteriovenous malformations. For example, in a dog with suspected insulinoma, abdominal ultrasound may or may not be able to locate a nodule/mass in the pancreas, especially in large breed dogs with intestinal gas; or a nodule(s) is found on ultrasound, but it cannot be distinguished from a benign lesion such as nodular hyperplasia. Additionally, nodal or liver metastases can also be difficult to detect or confirm on ultrasound. Neuroendocrine tumor types, such as insulinoma, often demonstrate intense arterial enhancement differing from the normal pancreatic parenchyma, which makes multiphase CT angiography a better diagnostic test than ultrasound, with the added benefit of better assessment of the regional lymph nodes and liver for metastatic disease (Mai et al 2008).

**Dynamic contrast-enhanced imaging (DCE)**
Measurement of perfusion characteristics derived from dynamic imaging of contrast material enhancement in the tumor after intravenous injection is possible using CT and MRI. This may have some use in differentiation of tumor types, though preliminary studies in canine brain tumors (McLeod et al 2009) showed overlaps in perfusion characteristics between histologic types. DCE imaging can be used as a surrogate marker of tumor oxygenation, which may affect the biologic behavior of a tumor and its radiation response. Decreased tumor oxygenation has been associated with an aggressive phenotype and with decreased local tumor control following irradiation. To this date, very few studies have investigated this feature in naturally occurring canine neoplasms (Van Camp et al 2000).

**Local tumor extension: MRI vs CT**

Determining exact tumor margins is important both for surgical and radiation therapy planning. CT and MRI imaging is in general better at defining tumor margins due to the cross-sectional nature and ability to obtain or reconstruct images in any plane, thereby facilitating linear and volumetric measurements. Definition of tumor margins can be improved in CT using intravenous iodinated contrast administration. MRI's inherent excellent soft tissue contrast provide even better delineation of tumor margins. For example, MRI was shown to be significantly more accurate than radiography, CT and scintigraphy to determine the margins of appendicular osteosarcomas in dogs (Wallack et al 2002). In another study from the same year (Davis et al 2002), MRI was less accurate than radiography and CT however in that study MRI was performed only on amputated limbs that had been refrigerated, which is known to decrease MRI signal and contrast (Ruder et al 2012). A recent pilot study (6 dogs) compared staging of canine nasal tumors with CT vs MRI (Lux et al 2017). MRI resulted in larger bidimensional measurements and tumor volume estimates, along with a higher likelihood of identifying meningeal enhancement when compared to CT imaging, all of which can be important for staging and treatment planning.

**Whole-body CT and MRI for staging**

Cancer staging is typically achieved through a combination of multiple imaging modalities and diagnostic testing that has been tailored to the cancer type and body region. This can be expensive, time-consuming and requires numerous pieces of equipment and expertise. Deriving cancer stage from a single imaging method is advantageous. Whole-body imaging has the potential to detect tumor burden throughout the body non-invasively. Contemporary whole-body imaging methods for cancer staging include MRI, CT, and positron emission tomography (PET).

In a recent study of whole-body CT in 61 dogs with hemangiosarcoma (Carloni et al 2019), skeletal muscle metastases were detected in 24.6% and all these dogs had also metastases in other sites such as lung, bone, or kidneys. In contrast, even though CT can detect pulmonary metastatic lesion of appendicular osteosarcoma better than radiography, whole body CT does not perform well in detecting bone metastases (Talbott et al 2017, Oblak et al 2013) and bone scintigraphy may be a better screening tool for that purpose (Oblak et at 2013).

Pilot studies have also investigated the use of 3T body MRI to detect bone marrow infiltrates in dogs with lymphoma and myelodysplastic syndromes with some suggestion that out-of-phase imaging (which normally suppresses signal in normal vertebral bone marrow with equal number of red (cellular) and fatty bone marrow) may be useful to detect hypercellular marrow in dogs with myelodysplastic syndrome (Feeney DA et al 2013).

**MRI: Liver-specific contrast agents**

Liver nodules are common in dogs and cats, however determining if they are benign or malignant is difficult as their imaging features are non-specific using traditional ultrasound, CT or MRI imaging. MRI can be performed using liver-specific gadolinium-based contrast agents (eg Multihance$^{TM}$), which are taken up by hepatocytes and excreted in the bile and can be used for determination of benign vs. malignant liver lesions (Yonetomi et al 2012, Louvet et al 2015). Normal liver parenchyma will accumulate these liver-specific contrast agents and will appear markedly hyperintense when scanning about 20min after injection. In contrast, due to their altered hepatocyte function, malignant lesions will not take-up contrast and therefore have 'negative

contrast' uptake and appear hypointense on a delayed "hepatocellular phase". Benign nodules due to their retained hepatocyte function, will behave like normal liver parenchyma on these delayed scans (i.e., should be isointense to the rest of the liver). Sensitivity for nodule detection is also better in the delayed phase, often showing more lesions than seen on the routine T2W and T1W post-contrast series.

**Diffusion-weighted MRI**

Diffusion-weighted magnetic resonance imaging (DWI) is a functional imaging technique that generates image contrast based on differences in water proton mobility within a voxel of tissue. Currently, the main application of DWI in veterinary medicine is in the context of brain ischemia where the acute cytotoxic edema resulting from ischemia causes focal reduction in water mobility (restricted diffusion), which can be readily identified with diffusion-weighted imaging. In people however, DWI has been extensively used and evaluated in cancer imaging. In tumor tissue, water mobility is limited because of the inherent high cellularity, leading to higher signal intensities on DWI images corresponding to lower diffusion coefficients on the calculated Apparent Diffusion Coefficient (ADC) map. This contrasts with the normal surrounding tissue where the water protons can freely move and generate high diffusion coefficients (unrestricted diffusion). The large microstructural differences between tumor and normal tissue may allow tumor detection at millimeter level. In people, ADC values may be decreased in highly cellular tumors such as lymphoma, medulloblastoma, and high-grade gliomas. Lower ADC values have been associated with higher grade tumors and poorer prognosis (Thoeny et al 2010, Kono et al 2001) and are used in predicting the progression of intracranial disease in humans. For example, primary central nervous system lymphoma with a low pretreatment ADC value was associated with a higher risk of progression and death (Barajas et al 2010). DWI MRI can also be used for treatment monitoring: increasing ADC values observed on serial MR examinations may indicate treatment response because responding neoplastic lesions undergo a reduction in cellular integrity with an increase in water mobility (Theilmann et al 2004, Kono et al 2001). Although controversial, ADC values may be lower in infiltrative (i.e., neoplastic) peritumoral brain edema than in lesions with vasogenic peritumoral edema, where both would have a similar appearance on other MRI pulse sequences; this could have implications in defining treatment field in radiation treatment planning for example (Hespel et al 2018). In people, DWI can be used to detect abdominal peritoneal spread of neoplasia as it significantly improves peritoneal tumor depiction, particularly for mesenteric/serosal disease (Dresen et al 2019). Although CT has a good accuracy for detecting liver and lung metastases, estimation of peritoneal carcinomatosis is suboptimal, due to its limited soft tissue contrast resolution. $^{18}$F-Fluoro-deoxyglucose positron emission tomography ($^{18}$F-FDG PET)/CT can only partially overcome this problem as its lower spatial resolution limits sensitivity, especially in small volume disease. Therefore, both techniques have limitations for treatment planning.

DWI can be used as a whole-body imaging technique, allowing assessment of primary tumor and metastases in one clinically time-efficient examination. For example, in people, whole-body MRI can identify skeletal metastases from prostatic carcinoma with higher sensitivity than bone scintigraphy and the same sensitivity as PET/CT (Shen et al 2014). Whole body MRI was also shown to be more sensitive than PET-CT in all regions except the skull, to detect intramedullary lesions of multiple myeloma and equally sensitive to PET-CT for extramedullary lesions (Chen et al 2019). The 2016 diagnostic criteria of the International Myeloma Working Group identified whole body-MRI and $^{18}$F-FDG PET/CT as the most sensitive imaging techniques for detecting

skeletal and extra-skeletal multiple myeloma invasion, respectively. Preliminary findings have also shown that whole body-MRI is better than CT and equal to PET/CT in staging aggressive lymphoma and Hodgkin lymphoma, whereas MRI is better for diagnosing bone marrow involvement in patients with low-grade lymphoma (Stecco[a] et al 2018). In prostate and breast cancer, whole body-DWI MRI is useful in assessing the response of bone lesions to therapy and detecting early non-responders, while in lung cancer, whole body-DWI MRI shows a similar sensitivity to $^{18}$F-FDG PET/CT in the detection of bone metastases; in bone metastases of thyroid tumors and melanoma, the whole body-DWI MRI shows a higher sensitivity when compared to $^{18}$F-FDG PET/CT (Stecco[b] et al 2018).

**Sentinel lymph node imaging**

Standard pre- and post-contrast CT performs poorly for lymph node staging. For example, a study of mandibular and medial retropharyngeal lymph nodes metastases detection with CT in dogs with oral and nasal cancer reported a Sensitivity of 11.6%, Specificity of 94.0%, Positive Predictive Value of 41.7% and Negative Predictive Value of 74.3% (Skinner et al 2018). Hence, more accurate methods to identify metastasized nodes for staging and targeted treatment are needed.

The sentinel lymph node is defined as the first lymph node within the lymphatic basin that drains the primary tumor. Therefore, examination of the sentinel lymph node is indicated to detect the presence of locoregional nodal metastasis. The sentinel lymph node may be the regional lymph node anticipated to be responsible for tumor drainage, but lymph nodes at unpredictable anatomical locations might also function as the sentinel lymph node. Indirect lymphangiography uses peritumoral contrast agent injection in four quadrants around the tumor immediately prior to imaging and can be valuable for the detection of sentinel lymph nodes in cancer patients (Beer et al 2018). A subcutaneous injection of 1 ml of nonionic iodinated contrast material (240-370 mg Iodine / mL) diluted 1:1 with sterile saline is performed at the palpable tumor edge (for a total injection volume of 2 ml) in a four-quadrant technique (total dose volume divided and split among the quadrants). The contrast agent can also be injected directly into the tumor tissue, although it is not recommended because of the risk of capsular damage and subsequent tumor cell seeding; it also has been shown to be less efficient at detecting lymphatic pathways and sentinel lymph nodes in dogs (Randall et al 2020). Lipid-based iodinated contrast media have also been used with good success with a potential advantage of longer persistence in the lymph nodes (Brissot et al 2016). After injection, the contrast material is drained towards the sentinel lymph node via the lymphatic system. Taking serial images within minutes after injection allows the delineation of these lymph vessels and sentinel lymph nodes. Several veterinary studies proved the successful implementation of CT lymphography in clinical canine patients with mammary cancer, anal gland sac cancer, head and neck cancer and tumors of various sites (Soultani et al 2016, Majeski et al 2017, Grimes et al 2017, Rossi et al 2018, Randall et al 2020). All studies used comparable CT imaging protocols including a pre-contrast CT study and sequential postcontrast images displayed in a soft tissue window, starting scanning within one minute after the injection. The contrast-filled afferent lymph vessels could be followed to one or more sentinel lymph nodes. In a study including 18 dogs with variable tumors on the head, indirect CT-lymphography was successful in identifying the sentinel lymph node(s) in 89% of cases (Grimes et al 2017) and 2 patients had more than one satellite lymph node. In a recent study of dogs with integumentary mast cell tumors, the satellite lymph node was identified with indirect CT lymphangiography in 90% of 20 dogs and differed

anatomically from the locoregional node in 28% of cases (Lapsley et al 2020); the identified satellite lymph nodes were metastatic in almost half of the cases.
Several studies have tried to evaluate if specific enhancement patterns of the satellite lymph node after indirect CT lymphangiography may help predict the presence of metastatic disease. Soultani et al (2016) studied 33 dogs with mammary gland tumors and described different lymph nodes opacification patterns: (1) homogeneous enhancement, (2) heterogeneous pattern and (3) missing contrast uptake into the satellite lymph node. The absence of opacification or heterogeneous opacification 1 min after contrast medium injection showed the highest sensitivity, specificity, and accuracy (93%, 100%, and 98.4%, respectively) for metastasis, while size and shape of the node were poor predictors. In images taken 1 min after injection, an absolute density value lower than 444 HU in the center of the node also provided significant sensitivity and specificity (93.8% and 75%, respectively). However, results are inconsistent, as another study of indirect CT lymphography in dogs with melanoma and mast cell tumor found that the satellite lymph node enhancement pattern (heterogeneous, homogeneous, or peripheral) was not associated with metastasis, nor was the attenuation value at 1 minute, 5 minutes, or the change in attenuation value (Grimes et al 2020).

**Dual energy CT**
Dual energy (DE) scanning implies the acquisition of CT data with 2 different X-ray spectra. The main advantage of DE scanning is that differences in material composition can be detected based on differences in photon absorption at different energies. This new application has been used in people for assessment of pulmonary perfusion and ventilation, characterization of renal and gall stones composition, and bone removal in CT angiography. Several other fields of clinical application are still under development. Material characterization is not the only advantage of DE scanning. With every DE scan, both low and high tube-voltage datasets can be separately evaluated. Scanning with a low tube voltage markedly increases attenuation of iodine (i.e. detection of contrast enhancement) but, unfortunately, image noise also increases. Higher tube voltage decreases image noise but at the expense of a lower detectability of contrast enhancement. The data of a pair of DE scan can be combined generating a weighted-average (WA) image dataset that combines the characteristics of the low- and high-peak voltage acquisitions according to the applied ‘weighting factor’. For example, at tube voltages 80 and 140 kVp, a 0.3-WA image dataset would be made up of 30% from the 80 kVp dataset and 70% from the140 kVp dataset. Using these strategies, compounded images with good signal and low noise can be obtained, while visualization of contrast-enhancing tissue is optimized, allowing for a better delineation of tumor margins and treatment planning (Tawfik et al 2012, Forghani 2019). Early studies are showing promising results using these techniques to improve detectability of tumor margins, increase the number of lesions detected as well as differentiate tumor tissue from normal anatomy which can have importance in radiation therapy planning (Agrawal et al 2014, Tawfik et al 2012, Forghani 2019).

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# Imaging for improved radiotherapy – planning, delivery, response monitoring

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Imaging is an integral part of radiation therapy and serves multiple purposes. Three dimensional images acquired before treatment will give us information about the size, shape and location of the target volume (tumor and locoregional extensions), as well as that of the any organs at risk (OARs) in proximity to this target. Hence, both target volume and OARs can be delineated in the images, resulting in 3D representations of these structures, as well as the spatial relationship between them. Furthermore, if the images are CT images, they will provide us with electron density information used in dose calculations. The images are then used as input to a treatment planning system that tries to optimize beam parameters to realize our treatment goal – to deliver a tumoricidal dose to the target volume while selectively sparing the adjacent normal tissues. The resulting dose plan is then delivered to the patient in a number of treatment fractions. Finally, tumor response is assessed by evaluating tumor size in repeated images, typically taken post-treatment.

As radiotherapy has moved towards increasingly conformal radiation dose distributions and as our methods for tumor imaging have improved, several challenges in imaging for radiotherapy have come to our attention. Firstly, multiple studies have shown that there can be large variations in target volume delineations between observers and that even intra-observer variability in target volume delineations can be significant (Weiss and Hess 2003). Secondly, the temporal stability of both target and OAR positions during the course of radiotherapy have come into question – both tumor regression and changes in normal tissues, e.g. due to weight loss during therapy, can lead to interfraction positional variations, while physiological motion can cause motion both during and between treatment fractions (Verellen et al. 2007). When the treatment plan is based only on pre-therapy images, both underdosage of tumors due to geographic misses and overdosage of normal tissues if these migrate into the original target volume may occur, potentially compromising our treatment goal. Furthermore, while new tumor imaging modalities have shown us considerable intratumoral heterogeneity in characteristics that may be relevant for radiotherapy, conventional anatomical images will not allow us to detect these, necessitating a uniform dose prescription to the target. Finally, follow up protocols in wich response assessment is only performed after the completion of therapy, render us unable to adapt therapy to changes, be it in anatomy or in tumor biology, occurring during the course of treatment.

The traditional approach to uncertainties in tumor location during the treatment course has been the addition of margins to the target volume (Burnet 2004). While this may give adequate tumor coverage, provided margins are large enough, it will also cause the inclusion of a larger volume of normal tissues within the target volume. As the dose to normal tissues frequently is the factor that limits the dose that can be delivered to the tumor, increasing the amount of normal tissues within the target volume may potentially compromise our ability to deliver a tumoricidal dose. Furthermore, in some

anatomical locations, the expected organ motion during therapy is such that prohibitively large margins on target volumes derived from pre-treatment images would be required, rendering radiotherapy unfeasible for these locations.

In the present talk, I will discuss how novel imaging strategies for treatment planning, delivery and monitoring may help address the above-mentioned challenges. Alternate modality or multimodality imaging, frequently providing biological or functional information, such as MRI, PET/CT or PET/MRI may lead to less inter- and intraobserver variability in tumor delineation (Buijsen et al. 2011, Queiroz et al. 2015), but questions on what is the optimal delineation method in these images still remain. I will present results from one of our recent studies on automatic tumor delineation in PET/CT images, investigating approaches ranging from simple thresholding methods to artificial intelligence, in the form of shallow machine learning and deep learning methods, demonstrating the superiority of the latter methods (Grøndahl et al. 2019a). I will also discuss imaging modalities aimed at identifying tumor characteristics relevant for the outcome of radiotherapy and demonstrate how information from these biological images can be incorporated into radiotherapy planning, creating biologically optimized treatment plans (Malinen et al. 2006). Furthermore, I will address radiomics based on pre-therapy images for predicting treatment outcome and present our recent results on how the addition of radiomics features may improve outcome predictions over those of clinical data alone, potentially paving the way for personalization of treatment (Grøndahl 2019b).

Image guidance and adaption of therapy, either through adaption of patient set up or through re-optimization and/or re-planning, go hand in hand. When imaging is introduced into the treatment room, margins that account for patient motion between and during treatment delivery can potentially be reduced. Currently, most commercial in-room imaging systems employ CT technology, with either kilo- or megavoltage photons and fan- or cone-beam geometries (Verellen et al. 2007). As the soft tissue contrast in these images may be less optimal than for diagnostic CT systems, corrections in patient set up are frequently performed using surrogates for tumor position, such as bony landmarks. These are non-deformable and easily identified and segmented in the images, facilitating automatization of registration of in-room images to those used for dose planning and consequent corrections of patient position. However, this strategy hinges on the correlation between surrogate and tumor movement. As an alternative strategy, we explored contrast-enhanced in-room cone-beam imaging and showed how the tumor could be well visualized in the resulting images (Rødal et al. 2010). We also demonstrated the feasibility of adaptive radiotherapy, both in terms on re-positioning and re-planning based on tumor position (Søvik et al. 2010).

Several techniques, such as gating, tracking and real-time adaptive radiotherapy planning, exist for dealing with motion of tumors and organs at risk during radiotherapy, i.e. intrafractional movement (Verellen et al. 2007). The development of these techniques has allowed substantial reductions in margins in certain tumor locations, making e.g. stereotactic body radiotherapy of certain liver metastases feasible (John et al. 2020). One modality that is particularly promising for image guided radiotherapy, is the Linac-MRI system that has been recently developed and is now commercially available (Lagendijk et al. 2014, Winkel et al. 2019). Here, fast MRI sequences with high soft tissue contrast enable both inter- and intrafractional imaging, and a fast,

adaptive planning workflow makes daily re-planning and re-optimization possible. Furthermore, MRI offers the possibility of biological imaging, with the potential for development of new biomarkers for assessment of tumor response during radiotherapy (Hunt et al. 2018, Pollard et al. 2017). Changes in such tumor characteristics could then be used for biology-guided adaption of radiotherapy. Previously, we have demonstrated the feasibility of adapting biologically optimized treatment plans to biological changes within the tumor during radiotherapy (Søvik et al. 2007). However, the logistics of combining daily out of room imaging with radiation therapy limits the clinical applicability of this approach. With the possibility of in-room biological imaging, biologically optimized adaptive radiotherapy could potentially become easier to implement in clinical practice. Furthermore, quantitative analysis of biological images may provide additional information to that available from mere visual inspection of images and could potentially offer additional insights into the underlying biology of tumor response to radiotherapy (Søvik et al. 2011, van Houdt et al. 2021). Such studies would also be more easily conducted when imaging and treatment delivery sessions can be combined.

In conclusion, the potential clinical benefit of conformal dose delivery can only be realized through integration with imaging modalities that allow monitoring of both tumors and organs at risk during the course of radiotherapy. At the same time, the conformal dose delivery is required to fully exploit the information from biological imaging in optimization and adaption of radiotherapy. Hence, developments in conformal radiotherapy and biological imaging go hand in hand and may together bring us closer to our treatment goal – to control the tumor while selectively sparing the normal tissues.

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# Adrenocortical tumors in dogs: building bridges between endocrinology and oncology

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Adrenocortical tumors (ACT) in dogs are becoming increasingly diagnosed in dogs. In the past, the most common diagnosis was a cortisol-secreting tumor (C-ACT) in dogs. Still, in the last decennium, hormonally silent adrenal tumors (S-AT) are being diagnosed often as well. Aldosterone-secreting ACT and sex-steroid secreting ACT are less common.

Cortisol-secreting adrenocortical tumors underlie 15 to 20% of cases of canine hypercortisolism (Cushing's syndrome). If no metastases are detectable, the treatment of choice for an ACT is adrenalectomy. Other or adjuvant treatment options include the steroidogenesis inhibitor trilostane, which will only reduce the clinical signs of hypercortisolism and has no effect on tumor growth, and the adrenocorticolytic agent mitotane, which can cause considerable side effects (Sanders et al. 2018).

Reported recurrence rates after adrenalectomy vary between 12 and 38%, which can be caused by metastases or regrowth of the ACT. Assessing the risk of recurrence after adrenalectomy is usually based on histopathology. However, the histopathological parameters that are primarily used in the assessment of canine ACTs often have high interobserver variability in human ACTs, and some studies did not observe a significant difference in survival times of dogs after adrenalectomy based on their histopathological diagnosis. To improve the reliability and prognostic value of histopathology in canine cortisol-secreting ACTs, we recently introduced a new histopathological scoring system: the Utrecht score. The Utrecht score was based on parameters with low intra- and interobserver variability and their association with the dogs' survival times. It includes assessment of the Ki67 proliferation index, the presence of necrosis, and the percentage of clear/vacuolated cytoplasm, and increasing Utrecht scores were significantly associated with shorter survival times. The score will still have to be validated in a different population and with the involvement of multiple (veterinary) pathologists. Moreover, it would be interesting to determine whether the score is also helpful for assessing S-ATs when they are of cortical origin (Sanders et al., 2019a).
In the most recent study on C-ACTs, the median survival time of 19 dogs with recurrence was 16.9 months (95% CI 10.8 – 49.3 months). If these dogs would have been classified as having a high risk of recurrence, they could have received adjuvant treatment post-operatively which might have improved their survival times. Moreover, if molecular markers that are associated with malignancy could be identified, this could give more insight into which molecular pathways are helpful to target for future treatment options.
Recent research by our group identified three genes of which high mRNA expression was significantly associated with poor survival: Steroidogenic factor-1 (*SF-1),* pituitary tumor-transforming gene-1 (*PTTG1)*, and topoisomerase II alpha (*TOP2A*). These genes are also potential treatment targets (Sanders et al. 2019b).

SF-1 is an orphan nuclear receptor that is important in both adrenal development and steroidogenesis. Interestingly, *SF-1* expression did not differ between adenomas and carcinomas, which might appear to contradict its prognostic relevance. However, this apparent contradiction could be related to the difference in SF-1 function depending on the cellular context: in differentiated adrenocortical cells, SF-1 mainly stimulates hormone production, whereas, in fetal adrenal development, SF-1 stimulates adrenal growth. Possibly, adenoma cells more closely resemble differentiated cells, and carcinoma cells more closely resemble fetal cells, in which high SF-1 expression might provide a specific growth advantage. Regardless of the mechanism, these results indicate that there might be room for improvement in the Utrecht score to refine prognostic classification.
The prognostic relevance of *SF-1* makes it an attractive therapeutic target. Compounds that can target SF-1 activity, called SF-1 inverse agonists, have been identified and inhibited cell proliferation and steroid hormone production in vitro in human C-ACT cells. We showed in a previous study that one SF-1 inverse agonist, compound #31, effectively inhibited cortisol production and SF-1 target gene expression in canine adrenocortical cells in vitro. If SF-1 inverse agonists are further developed for clinical use, this may have much potential to improve the prognosis of dogs with a C-ACT with high SF-1 expression.

PTTG1 is a securin that regulates sister chromatin separation during mitosis, and it plays a role in DNA repair, metabolism, senescence, apoptosis, and gene transcription. Several drugs have been shown to inhibit *PTTG1* expression, including BRAF, HDAC, Hsp90, and STAT3 inhibitors,[4]which could be interesting options to target PTTG1 in canine C-ACTs.

TOP2A is a nuclear enzyme that facilitates DNA unlinking, which is required for DNA replication and chromosome segregation. TOP2A is predominantly associated with proliferating cells, which makes it an interesting therapeutic target in cancer. Several TOP2A inhibitors are therefore successfully used in the clinic as anticancer drugs. The chemotherapy protocol that is most effective in human adrenocortical carcinoma is the combination of etoposide, doxorubicin, and cisplatin with mitotane (EDP-M). Of these, etoposide and doxorubicin are topoisomerase II inhibitors. In view of the prognostic relevance of *TOP2A* in canine ACTs, topoisomerase II inhibitors could be an interesting treatment to improve the prognosis of dogs with high intratumoral *TOP2A* expression.

One of the traditionally used drugs to treat ACTs is mitotane (o,p'-DDD). Its working mechanism is still a mystery, but we know that SOAT1 is its target. The expression of SOAT1 could therefore be indicative of the expected efficacy of mitotane. In canine adrenocortical tissue, the *SOAT1* expression was not significantly different in ACTs compared to normal adrenals. The novel SOAT-1 inhibitor ATR-101, is currently being studied as a potential treatment for humans with an ACC. It has recently also been administered to dogs with hypercortisolism, the majority having pituitary-dependent hypercortisolism, in which it significantly decreased ACTH-stimulated cortisol concentrations. ATR-101 could be an interesting treatment for dogs with an ACT, and further studies are warranted to determine whether there is a minimal degree of SOAT1 expression required for its effect.

In conclusion, most apparent molecular markers of malignancy of C-ACTs are *SF-1, PTTG1* and *TOP2A*. These findings can be used to refine prognostic prediction, but also offer substrate for future studies, where important prognostic markers could be targeted for new treatment options. If in the future drugs for multiple targets are available, treatment of dogs with a high risk of recurrence could be based on their C-ACT's molecular malignancy profile, thereby moving towards personalized treatment. Such studies in S-ATs are still lacking. When finding an adrenal mass incidentally, it is essential to screen for endocrine activity. In case of negative results, the diagnosis of S-AT is made. Hormonally silent adrenal masses are becoming an increasing problem in veterinary medicine, and the discussion about the treatment approach is ongoing. While a benign S-AT deserves no treatment, a malignant S-AT should be removed as soon as possible. Aspects of malignancy include: (1) size, (2) invasive growth, and (3) presence of metastasis. A size cut-off threshold for malignancy is unknown, but most of the authors agree that size of more than 2 cm is suggestive of malignancy. Invasion of the S-AT in the adjacent blood vessels is commonly seen in large adrenal masses, and there is no consensus whether this is a marker of malignancy or "just" an invasion of a benign mass to the surrounding tissue with little resistance. Metastases are the only straightforward sign of malignancy and are mostly detected in the liver, adjacent lymph nodes and/or lungs. The differentiation between benign or malignant S-AT by fine-needle aspiration biopsy (FNA) biopsy is not possible, and the procedure itself is not without risks. The main indication for FNA biopsy is a suspicion of metastasis in the adrenal gland.
At our institution, in a S-AT exceeding 2 cm, adrenalectomy is advised, while in masses of less than 2 cm through cut and no metastasis, a conservative approach is used. It includes diagnostic imaging (usually ultrasonography) initially every 3 months. In case of the deterioration of the adrenal structure, ingrowth of the adrenal mass and/or its increase in size, surgery can still be planned on time. If the disease is stable, re-checks are increased to every 6 months. Further research is needed to define the appropriate criteria for additional diagnostic testing or surgical intervention in dogs with S-ATs. Future studies om molecular characteristics and prognostic factors, comparable to research in C-ACTs, are ongoing and will hopefully lead to more objective approach.

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# Behind the mystery: understanding canine pheochromocytoma

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Pheochromocytomas (PCCs) are tumors that arise from the chromaffin cells in the adrenal medulla and are characterized by an overproduction of catecholamines such as epinephrine and norepinephrine. PCC has been called “the great mimic”, as its clinical signs and symptoms are often incorrectly attributed to other, more common conditions. Thus, the clinician's ability to consider the possibility of a PCC represents a crucial first step in the diagnostic process (Galac and Korpershoek 2017).

The majority of PCCs are unilateral, although approximately 10% of cases are bilateral. The size of a PCC generally ranges from a few mm to as large as 15 cm, and up to 56% of cases have local invasion into adjacent vessels. Invasive growth of the tumor is considered a sign of malignancy; metastases are present in approximately 20% of cases. Histopathology and immunohistochemical markers are currently not suitable for predicting a malignant disease course in PCC.

In the early beginning of the disease, the **clinical signs** of a PCC may be subtle, but in the later stage they become life threatening. A PCC is associated with the secretion of adrenaline and can lead to severe elevation of blood pressure, heart rate, discomfort, and even death. Hormone secretion from the tumour is episodic and unpredictable. The episodes of high catecholamine release may occur several times per day or only at intervals of weeks to months. Their severity varies from mild to life-threatening and may progress with time. The triggers for catecholamine secretion in dog are usually unknown.

Next to clinical signs related to hormone secretion, a PCC can also express tumor-growth related symptoms by invading the blood vessels, causing bleeding and metastasis.

An **endocrine diagnosis** of PCC consists of biochemical evidence indicating excessive catecholamine production and includes the measurement of plasma and/or urinary catecholamine metabolites, namely metanephrine (MN) and normetanephrine (NMN). The principle of measuring metanephrines is based on the intramedullary metabolism of catecholamines. The production and secretion of metanephrines in tumour cells is continuous and more accurately reflects tumour mass than the release of catecholamines, which is usually episodic.

In dogs, biochemical testing for PCC has only recently been initiated. Measurement of urinary catecholamines is performed in a single voided sample and their concentrations are expressed as ratios to the creatinine concentration in the same urine sample. Urinary sampling can be done in the hospital. Acidification of urine depends on the laboratory method used and is not always required. The NMN-to-creatinine ratio has a higher sensitivity than the MN-, epinephrine- and norepinephrine-to-creatinine ratios in the diagnosis of canine PCC. Urinary NMN ratio discriminated with no overlap between healthy dogs and dogs with PCC. This is not surprising as

canine PCCs produce predominantly norepinephrine, which is then metabolized to NMM (Salesov et al. 2015).

Corresponding to urinary measurements of NMN, measurement of plasma free NMN is superior to measurement of plasma free MN, and it was significantly higher in dogs with PCC compared to dogs with other adrenal tumours and healthy dogs. Sampling conditions are critical for reliable results. Blood should be collected in chilled tubes and plasma should be sent to the laboratory on ice. The availability of species-specific reference ranges is essential for the interpretation of the results.

Currently, there is no consensus about the use of plasma or urine, but there is a strong preference for NMN determination. The sensitivity and specificity of either test in dogs are unknown so far. The influence of drugs on NMN and MN levels has not been studied extensively in dogs so far. Based on the experience from human medicine, phenoxybenzamine can lead to false positive results and should be prescribed only after samples for endocrine testing have been taken.

The visualization of PCC with **diagnostic imaging** includes assessing their shape, architecture, size, margination of the mass, invasion of adjacent structures, and the symmetry of the adrenal glands.

Ultrasonography is the most used. There is no sonographic appearance that is typical for canine PCC. Most are unilateral and the contralateral adrenal gland is of normal size and shape, but about 10% are bilateral. Not unusually, PCC is diagnosed after an incidental adrenal mass is visualized during abdominal ultrasonography for an unrelated problem. Cortisol-secreting adrenocortical tumours remain the most challenging differential diagnosis. Both tumour types can be bilateral and even if a unilateral lesion is present, the atrophy of the contralateral adrenal gland is difficult to establish or may even not be apparent. Features that need to be evaluated during ultrasonography are possible invasion of surrounding structures and the presence of metastasis. It remains disputable, whether this relatively high percentage is a result of relative late diagnosis of a PCC because biochemical diagnosis was not generally available.

CT scan allows a more precise and complete evaluation of a tumour size, shape, and architecture, and is superior to detect vascular invasion. Another advantage of CT scanning is the screening for metastasis and the evaluation of comorbidities and among them, hypercortisolism on the first place. When performing a CT scan in a dog with a PCC, not only scanning of the abdomen, but also the thorax and head are advised.

While ultrasonography and CT and MRI have excellent sensitivity for detecting adrenal tumours, these anatomic imaging approaches lack the specificity required to unequivocally identify a mass as a PCC. The higher specificity of functional imaging—the test of choice is currently $^{123}$Iodine-labeled meta-iodobenzylguanide (MIBG) scintigraphy—offers an approach to overcome the limitations of anatomic imaging. This radiopharmaceutical competes with norepinephrine for uptake and storage in neurosecretory granules of catecholamine cells and thereby reveals functional medullary tissue. Its use in dogs has been reported only once. Position emission tomography (PET) with $^{18}$F-MIBG (fluorobenzylguanadine) is reserved for rapidly

growing, dedifferentiated tumours in which $^{123}$I-MIBG uptake is negative. There is still little experience with functional imaging of PCC in dogs because the technique is not widely available.

Percutaneous ultrasound-guided fine needle aspiration (FNA) biopsy is one of the most used techniques for the collection of samples for cytological analysis. Adrenal cytology is able to discriminate between the cortical and medullary origin with a diagnostic accuracy of about 87%, but distinguishing benign from malignant lesions is inaccurate. The risk assessment of the FNA of an adrenal mass has been evaluated in dogs with adrenal tumours, which were detected incidentally during a diagnostic imaging in dogs that were not suspected of having an adrenal disease. The masses included cortical and medullary tumours, that were either hormonally active or hormonally silent. The procedure was judged as relatively safe, and the most common complications were haemorrhage, ventricular tachycardia, and respiratory distress.

The clinical indication of performing the FNB of the adrenal gland in dogs suspected of a PCC is questionable, as the results of the cytology only provide the information of the origin of the tumour, but not about its secretory ability and malignant potential. Having in mind that the risk of performing the FNA in canine PCC has not been established in a large group of dogs, makes a biochemical diagnosis of a PCC is still the preferred option.

The preferred first-line **treatment** for canine PCC is an adrenalectomy, as removing the tumor will reverse the high catecholamine–related symptoms and prevent uncontrolled tumor growth. The surgery is a high-risk procedure and can be performed only at specialized facilities; in addition, presurgical treatment with the long-lasting alpha-adrenoceptor antagonist phenoxybenzamine is mandatory. On the other hand, if left untreated PCC can lead to catecholamine-induced conditions such as hypertension and tachycardia, as well as tissue mass–related consequences such as tumor rupture and abdominal bleeding.

In cases in which surgical removal is either not feasible or is prevented by a concurrent disorder or metastasis, medical treatment using tyrosine kinase inhibitors (TKIs) is an option. This treatment has been described in a small series of five dogs with PCC and has been translated from humans with inoperable PCC for whom TKIs appear to have a biological response of 60%.The recommended dosing regime is to administer 2.75 mg/kg body weight SID three times per week (Musser et al. 2018). Some oncologists prefer Monday-Wednesday-Friday schedule than every other day protocol. Treatment is associated with adverse reactions, such as diarrhoea, decreased appetite, lameness, muscle weakness, proteinuria, and hypertension. Among laboratory abnormalities anaemia, thrombocytopenia and neutropenia can occur, as well as increased ALT and creatinine. Dose interruptions and dose reductions may be needed during the treatment course depending upon the severity of clinical signs. The dose should be adjusted based on regular veterinary assessments during the first 6 weeks and approximately every 6 weeks, thereafter. After two weeks, blood examination with CBC and biochemistry is required to evaluate the safety of the drug. After six weeks, blood exam is combined with the diagnostic imaging. The treatment is continued only in stable presentation of the adrenal mass. Decrease of the adrenal mass has so far not been achieved. Treatment with toceranib phosphate provides a survival advantage in dogs in PCC. Progression free interval in dogs without metastatic disease was 28,

36 and 61 weeks. Survival with metastatic disease was 11 and 18 weeks. Toceranib phosphate can be combined with phenoxybenzamine, if indicated.

The background of treatment with toceranib phosphate is the blockade of the two critical receptors associated with angiogenesis, namely the vascular endothelial growth factor (VEGF) receptor and the platelet-derived growth factor (PDGF) receptor. These two receptors are part of a kinase signaling pathway but are also targets in the so-called pseudohypoxia signaling pathway. Activation of these pathways stabilizes hypoxia-inducible factor alpha (HIFα), which induces angiogenesis, apoptosis, proliferation, cellular and vascular invasion, metastasis, and increased energy metabolism, all of which are prerequisites for a tumor to become malignant. In brachycephalic breeds, chronic hypoxia may be the initial trigger that leads to the development of a PCC. Another factor that can activate pseudohypoxia is a mutation in genes encoding succinate dehydrogenase (SDH) family members. Mutations in *SDHA*, *SDHB*, and *SDHD* have been reported in dogs (Korpershoek et al. 2018).

Although canine PCC is considered to be a rare disease, precise data regarding its prevalence are unknown. Making an endocrine-based diagnosis of a PCC became possible only five years ago, and we now routinely diagnose a new case of canine PCC in our service nearly every week. This suggests that PCC is one of the most underdiagnosed conditions in dogs.

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# The science behind canine and feline pituitary tumors: novel approaches

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Pituitary tumors account for approximately 13% in dogs and 9% in cats of all intracranial tumors. Most frequently, they arise from the adenohypophysis, but other masses in the sellar region may include craniopharyngioma, Rathke's cleft cyst, metastasis or ingrowth of a different tumor, secondary neoplasm, hypophysis's, or pituicytoma. In dogs, the far most frequent tumor is corticotroph adenoma, and in cats somatotroph adenoma; therefore, the focus of this text lies on these two types.

**Corticotroph adenomas** originate from the TPIT lineage that express ACTH. Corticotroph adenomas can excessively secrete ACTH, which results in pituitary-dependent hypercortisolism (PDH), also known as Cushing's disease. PDH is one of the most common endocrine disorders in dogs, with an estimated prevalence of 1 in every 500 dogs. The chronic hypercortisolism results in severe multisystem morbidity, which becomes evident through clinical signs such as polyuria, polydipsia, polyphagia, central obesity, skin and muscle atrophy, hepatomegaly, progressive bilateral alopecia, panting, and systemic hypertension. In addition to the effects induced by hypercortisolism, the pituitary adenoma can induce space-occupying effects, which can result in neurological signs such as altered behavior, abnormal posture and gait, and cranial nerve deficits. Corticotroph adenomas have been described with other concurrent endocrine neoplasia in dogs, most often found with adrenal gland tumors.

The goals of treating canine hypercortisolism would optimally be to eliminate the source of either ACTH or autonomous cortisol excess, to achieve normocortisolism, to eliminate the clinical signs, to reduce long-term complications and mortality, and to improve the quality of life. Surgical removal of the causal tumor or radiotherapy are currently the only treatment options that have the potential to eliminate the source of either ACTH or autonomous cortisol excess. However, these options are not without risks, not widely available and not appropriate for every patient. Therefore, pharmacotherapy targeting the pituitary gland tumor, would be promising approach for the future. Currently, several options are available, but none of them was tested in a large population and evidence-based data are still lacking (Sanders et al. 2018).

Medical management of PDH would ideally target the pituitary tumor. Because dopamine (DA) and somatostatin (SST) both have inhibitory functions in the pituitary gland, the focus in research on pituitary-targeting drugs are three receptor subtypes: DA receptor subtype 2 (DRD2), and SST receptors subtype 2 (SSTR2) and subtype 5 (SSTR5). In canine corticotroph adenomas, the receptor subtype that is mainly expressed is SSTR2, while DRD2 and particularly SSTR5 are expressed at much lower levels (De Bruin et al., 2008). There are currently no pituitary-targeted drugs that are registered for use in canine PDH.

**Cabergoline** is a DA agonist that binds to the DRD2. In line with the moderate DRD2 expression in canine corticotroph adenomas, canine corticotroph cells responded only modestly to cabergoline in vitro. However, in vivo experiments showed that 43% of dogs with PDH responded well to treatment with cabergoline: the clinical signs of hypercortisolism diminished and the pituitary adenomas decreased in its size (Castillo et al., 2008).

**Pasireotide** (SOM230) is a multiligand SST analog that binds to the SST receptors 1, 2, 3 and 5. In 20 dogs with PDH, pasireotide decreased the plasma ACTH concentration and improved the clinical signs, while no severe adverse effects were observed (Castillo et al., 2011). In a recent study, pasireotide was administered to dogs with macroadenomas that were also treated with trilostane or mitotane. The pituitary tumor volume decreased in six out of nine dogs, and increased in the remaining three, while no neurologic signs or grossly apparent adverse effects were observed. An SST analog that has higher affinity for the SST2 than pasireotide could prove to be more effective in dogs.

**Octreotide** is an SST analog that binds to SSTR2 with high affinity, and to SSTR3 and SSTR5 with moderate affinity. In line with the high SSTR2 expression in canine corticotroph adenomas, octreotide significantly inhibited ACTH release in canine corticotroph cells in vitro and did so more effectively than either pasireotide or cabergoline. In humans, octreotide can cause gastrointestinal side effects, but this is less well documented in dogs. For other indications such as insulinoma, octreotide is sometimes used as adjunctive treatment to inhibit insulin secretion. However, its short duration time after subcutaneous injection limits its use. Recently, a new technology has been developed that increases the absorption of drug molecules through transient opening of the tight junctions of the gut epithelium, which can achieve therapeutic octreotide levels after oral ingestion in humans. An advantage of oral octreotide could be the lack of injection-related side effects, but there is a need for a strict twice daily fasted dosing regimen. Due to the high SSTR2 expression in canine corticotroph tumors, the availability of oral octreotide treatment could be an interesting treatment approach for dogs with PDH.

Another approach that is currently being developed is the use of **DA/SST chimeras**, which can cause SST and DA receptors to heterodimerize and generate a more effective hybrid receptor. This treatment approach seems very promising, and developments to produce an effective SSTR2/SSTR5/DRD2 chimera are ongoing (Culler, 2011)

To produce ACTH, the POMC is cleaved into multiple peptide hormones. The gene expression of POMC is regulated by many factors, including the transcription factors AP-1 and Nur77. **Retinoic acid** is an agent that regulates multiple cellular processes, including reducing the binding of these transcription factors to their DNA binding sites, ultimately inhibiting ACTH secretion. In 22 dogs treated with retinoic acid, investigators reported a decreased plasma ACTH concentration, decreased UCCR, resolved clinical signs, and decreased pituitary size (Castillo et al., 2006).

**Somatotroph adenomas** have been reported in at least 393 cats, making it the most reported pituitary tumor type in cats. Somatotroph adenomas that induce hypersomatotropism (HS) are most often detected in middle-aged to older cats. The

growth hormone (GH) excess can cause insulin resistance, which can result in diabetes mellitus. Recent reports suggest that HS occurs in 18% to 25% of cats with diabetes mellitus. In the largest study that screened for HS in diabetic cats to date, HS was suspected by the clinician in only 24% of cats that were found to have increased IGF-1concentrations. The current estimation is that 1 in every 800 cats has HS. Considering that hundreds of millions of cats live in this world while only 393 cases with somatotroph adenomas have been reported in literature so far, HS seems to be a highly underreported endocrinopathy. Although cats with HS were previously thought to always be diabetic, recent reports have shown that is not always the case. Clinical conditions other than diabetes mellitus that can raise suspicion of HS include hypertrophic cardiomyopathy-like disease, snoring, and abnormalities of the central nervous system.

The cause of the relatively high frequency of somatotroph adenoma formation in cats is not clear, but there has been some speculation on whether organ halogenated chemicals (OHCs) play a role. Cats with HS were found to have higher plasma OH concentrations than cats with diabetes mellitus without HS and healthy cats. These OHCs share some chemical characteristics with estrogens, which could potentially induce pituitary adenoma formation. In a rat somatolactotroph cell line, OHCs were recently reported to increase cell proliferation in a concentration-dependent manner. In addition, the aryl-hydrocarbon-receptor interacting protein (AIP) gene was found to have a single nonsynonymous single-nucleotide polymorphism (SNP) in 20%of studied cats with acromegaly. This SNP potentially affects the function of the AIP protein, which is thought to be a tumor suppressor, and can modulate the response to estrogens, androgens, and xenobiotics. The latter function is especially interesting, since this might be related to the OHCs. Environmental and genetic factors therefore potentially play a role in feline somatotroph adenoma formation (Schudder et al. 2018)

Treatment options for HS in cats include transsphenoidal hypophysectomy, radiotherapy, and medical treatment. Hypophysectomy can cure cats with HS, of which a high percentage also achieves diabetic remission. Radiotherapy can reduce or resolve the neurological signs by reducing the tumor size. Remission of HS or diabetes mellitus is, however, often not achieved.

For pituitary-targeted medical treatment options, **DA agonists** and **SST analogues** have been studied. SSTR1, -2, -5, and DRD2 were expressed in pituitary glands of cats with HS. The DA agonist cabergoline gave unsatisfactory results, while the multiligand SST analogue pasireotide has been shown to decrease IGF-1 concentration and increase insulin sensitivity in cats with HS and concurrent diabetes mellitus.

In conclusion, in dogs and cats, pituitary tumors are being diagnosed with increased frequency. Recent research in both conditions has unraveled some molecular targets for medical approach, however, they still need to be validated. With new in vitro testing modalities, like pituitary organoids and tumoroids, further steps in selecting the best treatment option or even a combination of two or three drugs can be selected before approaching testing in vivo.

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# Pituitary tumors in dogs and cats

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## Pituitary tumors

Pituitary tumours are common in dogs and are being increasingly recognized in cats. Pituitary tumours are usually classified as adenomas and should only be classified as carcinomas when there is evidence of metastatic spread of the tumour, which is rare. Despite the benign nature of most pituitary tumours, they can still compress or invade neighbouring tissues. Pituitary tumours can be functional (hormonally active) or non-functional (hormonally silent). In dogs, the most common pituitary tumour type is the corticotroph adenoma, which can cause pituitary-dependent hypercortisolism (Cushing's disease). In cats, the most common pituitary tumour is the somatotroph adenoma, which can cause hypersomatotropism (and acromegaly), and the second-most common is the corticotroph adenoma. A lactotroph adenoma has been described in one dog, while gonadotroph, thyrotroph and null cell adenomas have not been described in dogs or cats. Hormonally silent adenomas are likely underdiagnosed because they do not result in an endocrine syndrome. Tools used to classify pituitary tumours in humans, particularly immunohistochemistry for lineage-specific transcription factors, are likely to be useful to classify canine and feline pituitary tumours of unknown origin.

## Introduction to pituitary surgery

Transsphenoidal selective adenomectomy is the primary therapy for Cushing's disease in humans. The most common approach in humans is by the standard microsurgical submucosal transseptal transsphenoidal procedure using an endoscope or neurosurgical operating microscope. There are many virtues of the midline transsphenoidal approach. Most importantly, it is the least traumatic route of surgical access to the sella. The lack of visual scars, lower morbidity and mortality as compared with transcranial procedures, the necessity of only a brief hospital stay, the relatively brief recuperative period add to the procedure's appeal. More and more human pituitary surgeons employ the pure endoscopic endonasal transsphenoidal surgical approach for pituitary tumor removal using rigid endoscopes. The pure endoscopic approach in humans is facilitated by the air-filled sphenoid sinus that is only separated by a thin bony floor from the pituitary fossa. In addition to surgery of the pituitary fossa, this route is also chosen for extended skull base surgery.
In dogs the most common method of treatment for Cushing's disease (**Figure 1**) or pituitary-dependent hypercortisolism (PDH) today remains medical treatment with trilostane or less commonly mitotane (o,p'-DDD). However, medical therapy leaves the pituitary adenoma untreated. Also, it may be hypothesised that inhibiting production and/or release of glucocorticoids at the adrenal level, and thereby removing the chronic negative feedback exerted by the glucocorticoids at the pituitary level, may actually stimulate pituitary tumor proliferation and expansion. Also in recent years due to developments in the field of neurology and imaging, more and more pituitary abnormalities other than the corticotroph adenoma are recognized, such as clinically non-functioning adenomas (NFAs) creating a mass effect on adjacent neural

structures, pituitary cysts, pituitary apoplexy and other rare sellar disorders (e.g. lymphocytic hypophysitis).
At the Utrecht University transsphenoidal hypophysectomy was re-started in 1993 and has become an important addition in the management of Cushing's disease in the Netherlands (and for patients coming from other European countries). Until now 380 dogs and 30 cats have undergone pituitary surgery. Surprisingly, pituitary surgery in dogs and cats is, besides the Netherlands, only advocated in few other institutions (UK, Japan, USA, Italy). In dogs the main indications for pituitary surgery include pituitary corticotroph adenomas (causing Cushing's disease), debulking of clinically non-functioning pituitary macro-adenomas (causing diabetes insipidus or central neurological signs by the tumor mass effect) and occasionally sellar meningiomas. In cats the most common indication for pituitary surgery is hypersomatotropism (causing acromegaly) due to a growth hormone producing pituitary adenoma and clinically non-functioning pituitary adenomas causing a mass effect.

**Pituitary Imaging**
Magnetic resonance imaging (MRI) and contrast-enhanced computed tomography (CT) visualize the pituitary size and the surgical landmarks that are a prerequisite for pituitary surgery. Although MRI and CT (**Figure 2**) are both excellent modalities for imaging of pituitary masses, CT is preferred to low field MRI when pituitary surgery is a treatment option, because of better resolution with modern CT scanners and the ability to precisely define the bone surgical landmarks. Since the surgical landmarks are bone structures, they are more difficult to discern on MR images than on CT images. Low field (0.2T) MRI is not practical for imaging the pituitary gland and the surrounding bone structures. High field (1.5 and 3T) MRI is the pituitary imaging technique of choice in humans but is not routinely available in the veterinary field and still has to prove its value for pituitary imaging in dogs and cats. CT has proven to be practical, fast and accurate for detection of pituitary abnormalities. Modern 64- and 128-slice CT scanners usually allow fast contrast-enhanced spatial series producing transverse images from rostral to caudal through the skull and the pituitary fossa area. This also allows multiplanar reconstructions (MPR sagittal and axial) of the skull and the pituitary fossa. In addition to the spatial series, a dynamic CT series can be performed at the level of the fossa.
Surgical localisation of the pituitary gland in the various canine and feline skull types is dependent on the continuous visual assessment of typical bone features during the transsphenoidal approach and relating those, real-time, to the CT images in the operating room. CT allows for accurate and reliable planning of the surgical approach and with the present DICOM viewers and 3D reconstructed images, the approach can even be simulated in silico in the specific patient. CT should include the spatial series (contrast enhanced scanning from rostral to caudal through the skull), as well as dynamic contrast enhanced scanning at the level of the pituitary lesion. Afterwards, multiplanar reconstructions allow for viewing the pituitary tumor in coronal, sagittal and axial views. 3D reconstruction of the bony calvarium allows for pre-operative viewing of the sphenoid anatomy and planning of the burr hole. Important landmarks for the precise location of the burr hole are bone features that are visible or palpable in the approach and can be easily identified on CT in relation to the pituitary tumor: 1) hamular processes and their disappearance on coronal scans in relation to the presence (or absence) of pituitary tumor, 2) the shape of the sphenoid surface (ridge, flat or groove) on coronal scans, 3) separation between pre- and basisphenoid bone on the sagittal multiplanar reconstruction, 4) on the midsagittal MPR the approach to

the pituitary fossa is simulated with a straight line towards the centre of fossa. In mesocepalic and dolichol cephalic breeds this line will not intersect with the palatum durum, and will indicate the amount of bone that needs to be removed to expose the ventral surface of the fossa. However, in brachycephalic breeds this line may intersect with the palatum durum, in which case the bony palatum durum needs to be shortened first by burring, before continuing the approach to the fossa.
The bone landmarks for pituitary surgery have been well-described for dogs and are essentially the same for cats, but with small differences. In dogs, the pituitary fossa is generally localized at the level where the pterygoid hamular processes, marking the end of the pterygoid bones, can be palpated with the index finger through the soft palate and actually visualized when the soft palate has been incised and retracted. Next, when observing the sphenoid bone during pituitary surgery, when the mucoperiost has been reflected, the (single) sphenoid bone can easily be recognized by fissures separating the sphenoid bone in the middle from bilateral pterygoid bones on left and right side. In the sphenoid bone itself the presphenoid bone can be discerned from the basisphenoid bone by a vague remnant fissure. Also the basisphenoid bone can be recognized by its broad base in comparison with the small width of the presphenoid bone that extends rostrally up toward the vomer bone. The presphenoid bone usually has a clear defined ridge (more clear in the cat) that flattens out caudally towards the basisphenoid bone and even may continue as a groove caudally. It is these two landmarks, i.e. the hamular processes and the surface changes of the sphenoid bone, that can be followed on the contrast-enhanced CT spatial series and will tell the surgeon where to center the burr hole to directly approach the pituitary fossa. In addition to these landmarks, and specifically for the cat (and not the dog) the surgeon is aided with the presence of an air-filled sinus in the feline sphenoid bone that starts just rostral to the pituitary fossa. The sinus is easily recognized on CT in the transverse images but especially on the midline sagittal MPR reconstruction the pituitary fossa is nicely shown caudal to the sinus. Lastly, and invariably present or recognized on the sagittal view in CT and MRI, some other features may be seen that can help in determining the location of the pituitary fossa and thus marking the site for the burr hole: sometimes the separation between presphenoid and basisphenoid bone can be identified on the CT/MR image (and seen in surgery), and invariably a remnant embryonic craniopharyngeal canal can be identified on the image starting in the fossa and exiting from the basisphenoid bone which is seen to contain a small vessel during surgery.
The enhancement pattern of the neurohypophysis during dynamic contrast enhanced CT has been called the ‘pituitary flush’. The displacement, distortion, or disappearance of the pituitary ‘flush sign’ in dynamic CT examinations can be used to confirm left or right-sided lateralization of (micro)adenomas.

**Surgical Technique**
Pituitary surgical techniques include selective removal of the pituitary adenoma (adenomectomy), removal of the adenohypophysis (adenohypophysectomy), removal of a significant part of pituitary tumor mass in the case of a macro-adenoma (pituitary debulking), or complete removal of the pituitary gland including the tumor (hypophysectomy). Hypophysectomy in the dog and cat is performed by the midline transoral, transnasopharyngeal, transsphenoidal, microsurgical approach (**Figure 3**). The dog is placed in sternal recumbency (**Figure 4**) and the upper jaw is fixed to a padded metal bar attached to the side of the operating table. The head and neck are supported by a vacuum cushion and care is taken not to hyperextend the neck, for

which the cat is particularly vulnerable (risk of brain stem necrosis). The head is fixed with elastic bandage and tape to the metal bar and the lower jaw is gently reflected downwards with elastic bandage (**Figure 4**). The cuffed tracheal tube is taped beneath the tongue to the lower jaw and reflected laterally out of the surgical field (to the right side of the dog for a right-handed surgeon). The surgeon is positioned in front of the operating table, facing the dog (**Figure 4**). The table is tilted at a 40º angle, which gives the surgeon an unobstructed view of the oropharynx almost perpendicular to the plane of the sphenoid bone. Following incision and retraction of the soft palate, access to the pituitary fossa is obtained with a burr. An operating loupe (**Figure 4**) or videoscope is used to provide magnification. Bone (Love Kerrison) punches are used to enlarge the opening created in the inner cortical lamina of the sphenoid bone. Following incision of the dura mater, the pituitary adenoma is extracted through the dural opening using fine neurosurgical grasping forceps and suction. In most cases the complete adenohypophysis is usually affected by the tumor and there is no sharp definition between adenoma and normal pituitary tissue. Unlike humans with Cushing's disease, well-defined pituitary (micro)adenomas are rare in dogs and cats. Completeness of hypophysectomy is assessed by the surgeon during surgery by the following criteria: (1) unobstructed view of the hypothalamus and the opening to the third ventricle, (2) absence of pituitary remnants on careful exploration of the hypophyseal fossa and the dorsum sellae with a ball-tipped probe, and (3) comparing the excised pituitary tumor volume to the pituitary tumor volume on CT/MRI. The pituitary fossa can also be inspected for pituitary tumor remnants using rigid endoscopes, e.g., and endoscope with a diameter of 2.7 mm and a 30 degree viewing angle. In dogs with giant (>2 cm) pituitary adenomas, the aim is to remove as much of the tumor tissue as possible within the safe margins of pituitary surgery to reduce the mass effect (debulking).

Postoperative intensive care includes close monitoring of vital functions, plasma electrolytes (sodium and potassium), plasma osmolality, and central venous pressure. Oral water intake is encouraged as soon as possible. Postoperative medication includes antibiotics and analgesics. Hormone replacement consists of hydrocortisone (1 mg/kg IV every 6 hours) and desmopressin (Minrin 0.1mg/ml), a vasopressin analogue (administered as a drop (5 µg) into the conjunctival sac every 8 hours for 2 weeks) or 0.5 to 1 tablet (0.1 mg) 3 times daily. When the dog has resumed eating and drinking, oral replacement therapy is started: cortisone acetate (1 mg/kg every 12 hours) and thyroxine (15 µg/kg every 12 hours). Over a period of 4 weeks the dose of cortisone acetate is gradually tapered to 0.25-0.5 mg/kg every 12 hours. Desmopressin is administered for 2 weeks, 1 drop into the conjunctival sac every 8 hours (or 1 tablet every 8 hours) after which is gradually tapered guided by drinking volume.

**Results and Complications**

In the largest cohort of dogs reported in 2016 (306 dogs), 91% of dogs were alive four weeks after surgery and remission was confirmed in 92% of these dogs. The median survival time was 781 days, median disease-free interval was 951 days. Over time, 27% of dogs developed recurrence of hypercortisolism after a median period of 555 days. Dogs with recurrence had significantly higher pituitary height/brain area (P/B) ratio and pre-operative basal urinary corticoid-to-creatinine ratio (UCCR) than dogs without recurrence. Survival time and disease-free interval of dogs with enlarged pituitary glands was significantly shorter than that of dogs with a non-enlarged pituitary gland. Pituitary size at the time of surgery significantly increased over the 20-year study period (1993-2013) at the same institution. Although larger tumors have a less favourable prognosis, outcome in larger tumors improved over time due to increased

experience with debulking of macro-adenomas, improvements on anesthesia protocols and postoperative intensive care.
Median survival time of the 300 dogs for which follow-up information was available was 781 days (range, 0–3808 days; Fig 3). Estimated 1-year survival rate was 86% (95% confidence interval [CI], 82–90%), estimated 2-year survival rate was 79% (95% CI, 73–85%), estimated 3-year survival rate was 74% (95% CI, 68–80%), estimated 4-year survival rate was 72% (95% CI, 66–78%) and estimated 5-year survival rate was 64% (95% CI, 56–72%). Disease-free interval was analysed for 257 dogs with confirmed remission of hypercortisolism after surgery. Median disease-free interval was 951 days (range, 31–3808 days). Estimated 1-year, 2-year, 3-year, 4-year and 5-year disease-free fractions were 89% (95% CI, 85–93%), 79% (95% CI, 73–85%), 74% (95% CI, 68–80%), 64% (95% CI, 56–72%) and 57% (95% CI, 49–65%), respectively. Treatment failures included postoperative mortalities (= death within 4 weeks after surgery irrespective of the cause of death, 27/306 dogs or 8.8% of dogs), and residual disease or incomplete hypophysectomy (16/279 dogs, 5.5%).
In another study the main non-life threatening postoperative complications after hypophysectomy were reduction in tear production (31%) and prolonged diabetes insipidus (53%). Tear production restored to normal values in 79% of the affected dogs over a median period of 9 weeks. Diabetes insipidus occurred more frequently in dogs with enlarged pituitaries than in dogs with non-enlarged pituitaries and was permanent in 22% of the dogs.
The results of hypophysectomy compared favourably with those of 129 dogs treated with o,p'-DDD in the same institution in another time frame. With longer follow-up time, hypophysectomy leads to better results than o,p'-DDD treatment.

**Adrenocortical Function after Hypophysectomy**
Adrenocortical function after hypophysectomy can easily be measured using the basal urinary cortisol/creatinine ratio (UCCR) in samples collected at home. When the patient leaves the hospital, usually 3 days after surgery, owners receive tubes for urine collection at 2 weeks, 8 weeks, 6 months, and 1 year after surgery. Thereafter yearly assessment of adrenocortical function is advised. Urine samples are collected at home when the dog is 24 hours free of cortisone medication. The early (<8 weeks) UCCR has prognostic value when considering long term survival and disease free fractions. In dogs with early postoperative UCCR $< 5 \times 10^{-6}$, the survival and disease free fractions are greater than in dogs with early postoperative values between 5 and $10 \times 10^{-6}$.

**Pituitary Surgery in Cats**
The main indications for transsphenoidal hypophysectomy in cats are: hypersomatotropism (acromegaly) caused by a GH-cell (somatotroph) pituitary adenoma and clinically non- functioning adenomas (creating a mass effect) and rarely Cushing's disease caused by an ACTH-cell pituitary adenoma. Hypersomatotropism and Cushing's disease in cats are usually accompanied by diabetes mellitus requiring insulin administration. Transsphenoidal hypophysectomy has a much higher morbidity and mortality in cats with Cushing's disease than in dogs and therefore hypophysectomy is not considered the first option of treatment for Cushing's disease in cats. However, for hypersomatotropism in cats the situation is the opposite.
In cats with acromegaly with concurrent insulin-resistant diabetes mellitus, hypophysectomy has an excellent prognosis resulting in disappearance of diabetes mellitus, discontinuation of insulin administration within 1 to 4 weeks after surgery, and

normalisation of GH and IGF-1 levels. In a cohort of 25 cats with spontaneous hypersomatotropism the median postoperative hospital stay was 7 days (range, 3-18 days). One cat died within 4 weeks of surgery. Median plasma GH concentration decreased significantly from 51.0 ng/mL (range, 5.0-101.0 ng/mL) before surgery to 3.8 ng/mL (range, 0.6-13.0 ng/mL) at 5 hours after surgery. Remission of hypersomatotropism, defined as normalization of plasma IGF-1 concentration, occurred in 23/24 cats (median, 34 ng/mL; range, 14-240 ng/mL) and 22/24 cats entered diabetic remission. Median survival time was 1347 days (95% confidence interval, 900-1794 days; range, 11-3180 days) and the overall 1-, 2-, and 3-year all-cause survival rates were 76%, 76%, and 52%, respectively. All together the beneficial outcome of hypophysectomy in cats with hypersomatotropism was marked by low death rate and a high percentage of diabetic remission and definitive cure.

## Further Reading


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**Figure 1. Left**: dog with Cushing's disease due to a pituitary corticotroph adenoma. The dog has a thin hair coat. **Right**: the same dog at 2 months after transsphenoidal hypophysetomy; the hair coat has completely regrown.

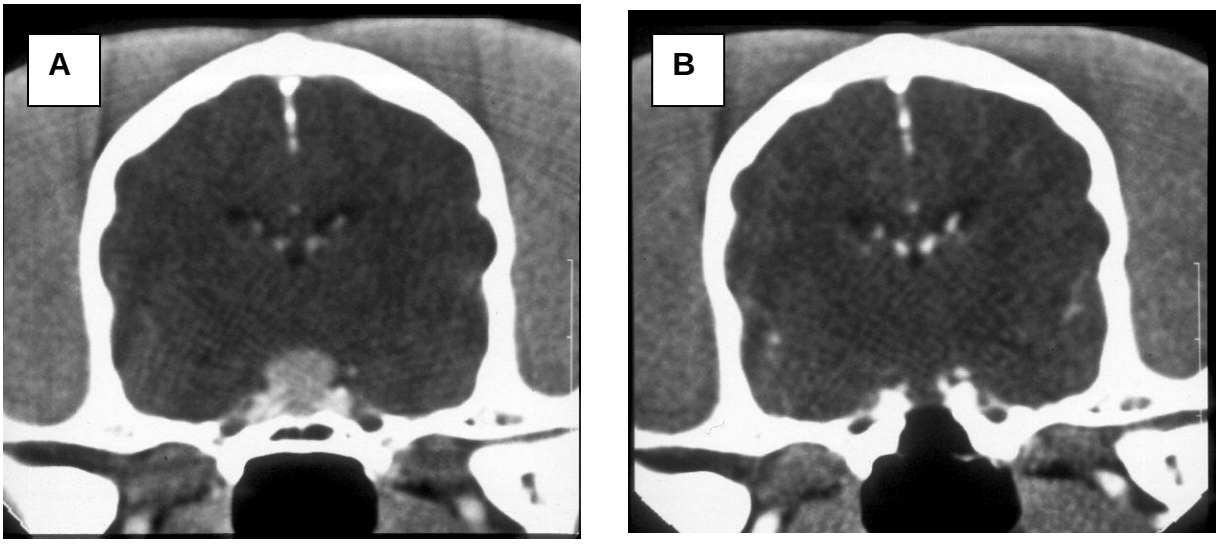


**Figure 2.** Transverse contrast-enhanced computed tomography of the skull of a dog with Cushing's disease due to a pituitary adenoma, before (**A**) and 8 weeks after transsphenoidal hypophysectomy (**B**).

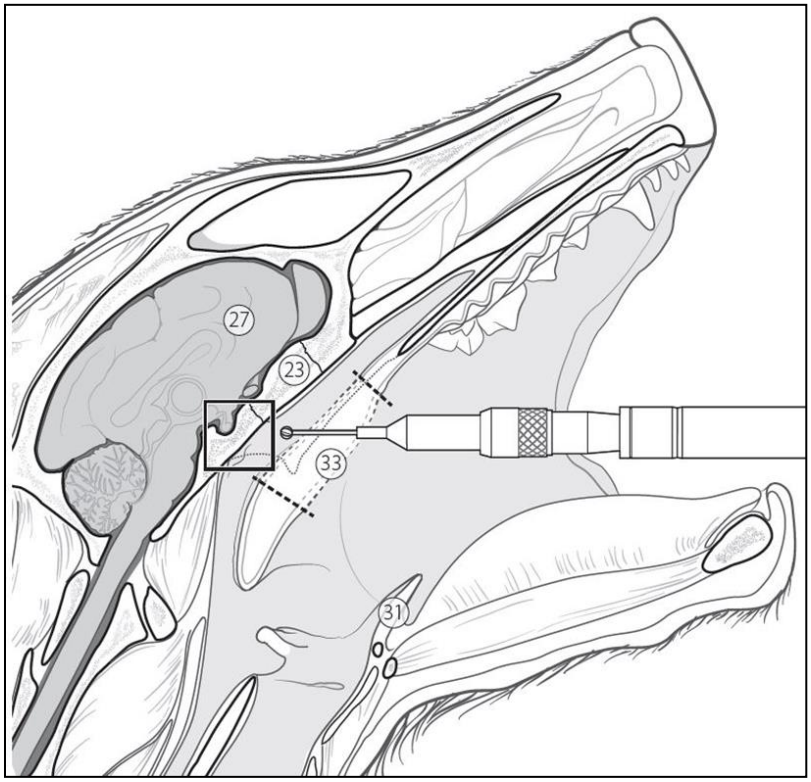


**Figure 3.** Sagittal schematic view of the canine skull for the transsphenoidal approach through oro- and nasopharynx that gives access to the pituitary fossa at the skull base. The rectangle points highlights the pituitary fossa containing the pituitary gland.

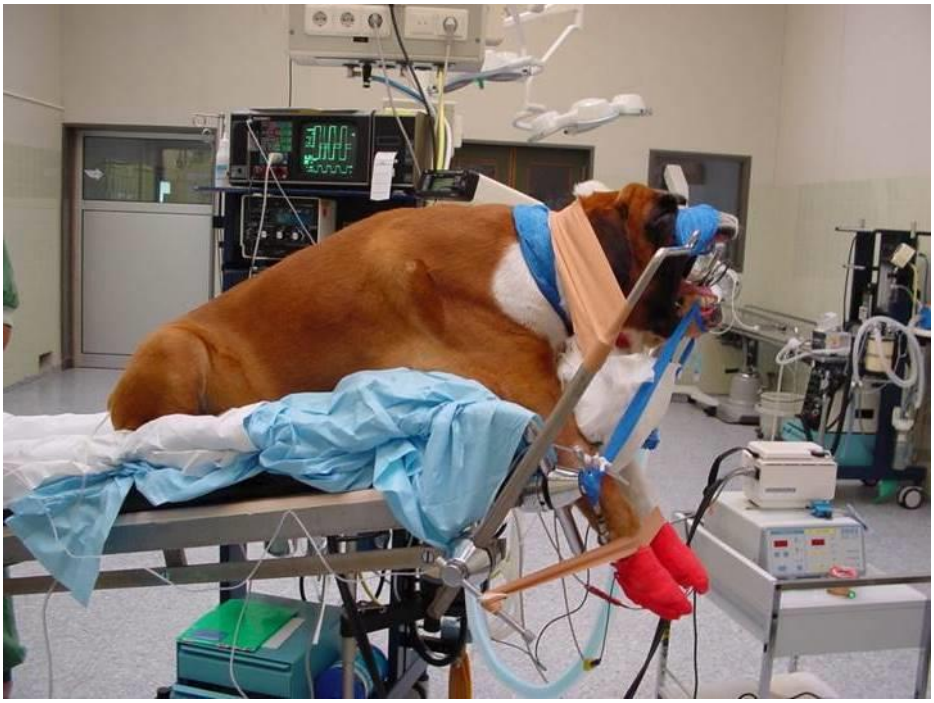

**Figure 4. Top**: Positioning of the dog for transsphenoidal hypophysectomy. The maxilla is resting on a metal bar, the mandibula is slightly retracted ventrally, and the skull is fixated with tape to the frame and the neck is supported. **Bottom**: The surgeon is sitting facing the dog and operates with magnification (operating loupe).

# RESIDENT WORKSHOP - Angiogenesis and Cancer

# Tyrosine-kinase receptors and their role in angiogenesis and tumour progression

Barbara Kitchell

## Can we effectively target angiogenesis? Available molecules, their applications and potential toxicities

Barbara Kitchell

# Vessels and cancer: a complex relationship

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*The cancer ecosystem*
The tumor microenvironment is formed by a complex combination of tumor and stromal cells, extracellular matrix (ECM), and secreted factors, thus perfectly fitting in the definition of an ecosystem. Alteration of the gene expression of tumor cells provokes a disruption in the normal tissue homeostasis, favoring the secretion of certain molecules (cytokines, growth factors, etc.) that recruit stromal cells. Cells composing the tumor stroma are cancer-associated fibroblasts (CAFs), endothelial cells, pericytes, adipocytes, and immune cells, including monocytes, macrophages, lymphocytes, and dendritic cells (DCs), among others. These cells are enclosed in heterogeneously deposited ECMs and are affected by changing biophysical parameters including oxygenation and pH. [19]

*The angiogenic switch*
An essential step required for tumor progression and propagation is called the angiogenic switch, a discrete point in time in which the balance between pro and anti-angiogenic factors tilts towards a pro-angiogenic outcome.[1] Pro-angiogenic gene expression is increased by physiological stimuli, such as **hypoxia**, which results from increased tissue mass, and also by oncogene activation or tumor-suppressor mutation. The angiogenic switch can occur at different stages of the tumor-progression pathway, depending on the tumor type and the environment.[1]
It is important to underline that both benign and malignant neoplasms gain angiogenic potential as they grow in size and acquire additional vascularization. Therefore, the angiogenic switch is often mistaken as a sign of malignancy. Rather, angiogenesis should be viewed as an" organizing principle" that is required to obtain a gain in tissue mass in a variety of diseases.[4]

*The role of hypoxia*
Oxygen plays a central role in vascular growth, and VEGF release from hypoxic tissues is a key event in regulation of angiogenesis. For tumors to grow beyond 1-2 $\mu m^3$ they need a continual supply of blood to supply nutrients and oxygen to overcome hypoxia and starvation. It is known that proliferating cells consume oxygen five times more than quiescent cells. Hypoxia of tumor cells will occur if the tumor grows beyond the maximum distance of diffusion from local vessels around 150 $\mu$m.[14] Cells deficient in oxygen produce **hypoxia-inducible factor (HIF)-1α**, a transcription factor responsible for upregulation of a multitude of angiogenic factors in response to hypoxia, among which is VEGF. VEGF secretion, induced by HIF-1α among other factors, strongly promotes angiogenesis; as explained later, tumor vessels are tortuous and abnormal, leading to turbulent blood flow, shear stress of red blood cells, leading to uneven distribution of oxygen within the tumor. This leaves a large number of neoplastic cells in a hypoxic state, leading to further VEGF secretion, setting up a loop promoting the formation of more abnormal vessels and leading to increasing microscopic regions of hypoxia.[14]
Hypoxia-inducible factors are heterodimeric transcription factors composed by a constitutive β-subunit and an oxygen-sensing α-subunit. Hypoxia-inducible factor-1α,

together with its ortholog -2α, are considered master regulators of the response to cellular hypoxia, being involved in all adaptive responses to low oxygen, from embryonic development to physiologic and pathologic conditions in adulthood. HIF-1α binds to hypoxia-response elements (HREs) and activates a number of hypoxia-response genes not only including VEGF, but also stromal derived factor 1 (SDF1), angiopoietin 2 (ANGPT2), placental growth factor (PGF), platelet-derived growth factor B (PDGFB), and stem cell factor (SCF) as well as genes involved in metabolic adaptation.

*Tumor versus physiologic angiogenesis*

During embryonic **vasculogenesis**, blood vessels are formed *de novo*, from endothelial-cell precursors (**angioblasts**) that assemble into a primary capillary plexus. This primitive network then differentiates, and new blood vessels sprout and branch from pre-existing capillaries in the process of **angiogenesis**.

The vasculature is usually quiescent in the adult; the few adult tissues that do require ongoing angiogenesis include female reproductive organs, organs that are undergoing physiological growth (e.g. the endometrium) or injured tissue.

*Mechanisms of tumor blood vessels formation*

Mechanisms of tumor blood vessels development include sprouting angiogenesis, inussusceptive angiogenesis, vasculogenesis from endothelial progenitor cells, vasculogenic mimicry, co-option, and trans-differentiation from stem-like cells.

*All these mechanisms are not mutually exclusive: they are interlinked, participating concurrently in physiological as well as in pathological angiogenesis.*

- *Sprouting angiogenesis*

By this mechanism, tumor cells induce their own vascularization from pre-existing host capillaries. Sprouting angiogenesis occurs through defined steps: a) Detachment of pericytes and dilation of blood vessels; b) endothelial cells migrate through the ECM in the perivascular space, following angiogenic stimuli produced by tumor cells; c) proliferation of endothelial cells; d) behind the migration column, endothelial cells adhere to each other and create a lumen through anastomosis.

Within this process, the angiogenic sprout is composed of endothelial cells playing different roles. Studies on the early postnatal retina show evidence that an endothelial “tip cell” guides the sprouting

Through filopodial extension from specialized endothelial cells situated at the tips of the vascular sprouts; tip cells are guided by VEGF. Beneath the endothelial cell tip is the “stalk” in which VEGF-guided proliferation of endothelial cells occur.[5]

- *Intussusceptive angiogenesis*

Also called non-sprouting or splitting angiogenesis. Through this mechanism opposite walls of capillaries start to migrate to each other, an intraluminal pillar develops, and the cellular junctions of the opposing endothelial cells are rearranged. Subsequently, further growth of the pillar leads to spitting of the blood vessel into two new vessels. Three different forms of intussusceptive angiogenesis are known[15]:

- *Vascuolgenesis*

This process is the main mechanism of *de novo* vessel formation in the normal embryo. It does not occur in adults, but can be “reactivated” in cancer. The process occurs through recruitment of **endothelial progenitor cells (EPC)** from bone marrow. In mice, progenitor cells differentiate and assemble into clusters called blood islands, as early as embryonic day (E) 6.5–7. A subset of cells located at the perimeter of the blood islands, termed **angioblasts** give rise to precursors for endothelial cells, while

those at the center differentiate to hematopoietic cells. Vasculogenesis in tumors is mediated by recruitment of EPCs (CD133+/CD34+/Tie-2+) or bone marrow–derived hematopoietic cells, resulting in the formation of new vessels to support tumor growth. Vasculogenesis is initiated by crosstalk between tumor cells and EPCs in the bone marrow. [10]

- *Vasculogenic mimicry*

Vasculogenic mimicry(VM) refers to the ability of cancer cells to organize themselves into vascular-like structures for the obtention of nutrients and oxygen independently of normal blood vessels or angiogenesis.[3] VM is characterized by the presence of non-endothelial, ntoplastic cells forming vascular structures containing fluid and/or erythrocytes. VM has been described in several human cancers and associated with pluripotent 'stem' cell marker expression, aggressive disease and poor patient outcomes with emerging evidence of VM involvement with tumour dissemination and metastasis.[3,17] Ever since its first report by Maniotis in 1999, the existence of VM has been an extremely contentious issue. The overwhelming consensus of the literature suggests that VM is frequently observed in highly aggressive tumors and correlates to lower patient survival. Nevertheless, the demonstration of VM is based on the positivity of vessels for PAS and negativity to EC-markers, which is by no means universally accepted. [17]

- *Co-option*

Vessel co-option (or vascular co-option) is a mechanism in which tumors obtain a blood supply by hijacking the existing vasculature and tumor cells migrate along the vessels of the host organ. The mechanism of co-option is well described in human glioblastoma multiforme where co-option is used by the tumor not only to survive but also to spread and represents the main reason for failure of anti-angiogenic treatment.[13] Vessel co-opting tumours can be discriminated from angiogenic tumours by specific morphological features. These features give rise to distinct histopathological growth patterns that reflect the interaction of cancer cells with the microenvironment of the organ in which they thrive.[9]

- *Trans-differentiation of cancer stem-like cells*

Trans-differentiation of cancer stem cells to endothelial cells and vascular smooth muscle-like cells, giving rise to neo- vascularization, has been reported in several tumor types. It has been demonstrated that in glioblastomas that the stem-cell-like CD133+ fraction includes a subset of vascular endothelial-cadherin (CD144)-expressing cells that show characteristics of endothelial progenitors capable of maturation into endothelial cells. [18]

*The mechanisms of angiogenesis differ among tumor types: the example of human glioma*

Astrocytomas first acquire their blood supply by co-opting existing normal brain blood vessels without the necessity to initiate angiogenesis. They instead grow along blood vessels, without a tumour capsule, eliciting an invasive character. When grade III astrocytomas progress into glioblastomas (GBM or grade IV astrocytoma), they become hypoxic and necrotic, partly due to vessel regression and increased tumour-cell proliferation. These conditions, in turn, induce formation of new blood vessels (angiogenic sprouting) that supply the tumour with the necessary metabolites. In fact, glioblastomas are partly defined by the appearance of proliferating endothelial cells and a high blood-vessel density that distinguishes grade IV tumours from the lower-grade astrocytomas.[1,8]

*Differences between normal and neoplastic vessels*

Normal vessels display an organized and hierarchical branching pattern of arteries, veins, and capillaries. In healthy vessels, endothelial cells are supported by basal membrane and pericytes coverage and they are tightly connected by stable cell-cell junctions. By contrast, tumor vessels are morphologically and functionally different from normal vessels. In response to persistent and imbalanced expression of angiogenic factors and inhibitors, tumor vessels display an unorganized network lacking a hierarchical vessel division. Moreover, tumor vessels are characterized by reduced blood flow, endothelial cell sprouting, disruption of endothelial cell junctions, loss of pericytes coverage and increased vessel leakiness resulting in increased tissue hypoxia and intravasation of tumor cells. In addition, tumor endothelial cell basal membrane is abnormal, including loose associations with endothelial cells and variable thickness.

*Lymphangiogenesis and lymphatic remodeling*

Lymphatic remodeling (LR) is the alteration to the structure and morphology of lymphatic vessels that are associated with cancer. LR Include lymphangiogenesis as well as lymphatic enlargement. The enlargement of lymphatics, which can occur via proliferation of lymphatic endothelial or other non-proliferative mechanisms.

VEGF-C and VEGF-D that signal through VEGFR-3 are essential for the development of lymphatic cells.

VEGF-C has been shown to be essential for the development of lymphatic vessels in embryos. In cancer, both VEGF-C and VEGF-D expression is correlated with lymphatic metastasis and in parallel, inhibition of VEGF-C and VEGF-D binding to the receptor has suppressed lymphatic metastasis. [16]

In analogy to blood vessels, lymphangiogenesis is stimulated during cancer growth. The mechanisms through which lymphatic vessels expand and proliferate are less characterized. It has been shown that lymphatic sprouting is the main mechanism of lymphangiogensis, while there appears to be no involvement of bone marrow-derived endothelial progenitors. [7]

The role of lymphatic is also well established in the metastatic process. The entrance of neoplastic cells in lymphatic vessels is favored by the presence of a fenestrated wall; moreover, neoplastic cells have better chances of survival in lymphatic vessels due to the presence of lymph, which has the same composition of the interstitial fluid, and due to the presence of low pressure flow, that causes minimal shear stress.[12,16]

Neoplastic cells access through lymphatics

*Contribution of angiogenesis to metastasis*

Angiogenesis and metastasis are intrinsically connected. Tumor angiogenesis enables tumor cell invasion and dissemination and favors the creation of new secondary tumor ecosystems at metastasized sites. VEGF-mediated effects provide a wide vascular area for intravasation of tumor cells as well as increase vascular permeability and basement membrane degradation, favoring escape of neoplastic cells. Pericytes help to control the patency of capillary lumens and are therefore essential for tumor cell dissemination. There is a strong line of evidence that increased tumor microvascular density correlates with increased metastatic potential. [2]

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# Methods to evaluate angiogenesis and vascularization in canine and feline patients

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Routine histology with H&E stain can be sufficient to evaluate certain features of tumor vascularization. The presence of lymphovascular invasion is routinely assessed in tissue samples to predict the presence of metastasis. Vascular invasion is also one of the criteria included in a few histological grading systems (e.g. grading of feline mammary carcinomas,[8] proposed grading of feline osteosarcoma[3] ).Moreover, the morphology of vessels can be evaluated with conventional histology; in fact, certain tumor types show vascular changes that may be associated with malignancy, for example, glomeruloid microvascular proliferation has been observed in choroid plexus tumors in dogs analogous to the same feature identified in human gliomas .[9]

Immunohistochemical markers are routinely used to evaluate blood and lymphatic vessels, both their presence, their amount and distribution. Several markers are available for human tissues, but only a small proportion has been tested and validated in dogs and cats. **CD31** (PECAM-1) is one of the most used endothelial marker in veterinary pathology.[2,5] CD31 is expressed in endothelial cells and leukocytes, as well as platelets. CD31 is the major constituent of endothelial intercellular junctions, playing a role in leukocytes trafficking, and transendothelial migration of leukocytes. The best alternative, and equally used, to CD31 is **von Willebrand Factor** (or Factor VIII), that is thought to be more specific for endothelial cells as it doesn't label leukocytes. vWF is a protein stored in Weibel-Palade bodies that mediates platelet adhesion to endothelial cells.

Both CD31 and vWF are used to assess **intratumor microvessel density (iMVD**). This method was firstly proposed by Weidner et al., in 1991, and is based on the assessment of the number of vessels within a defined area, that is expected to be a representation of the whole tumor vascular density. The method has been used in a wide range and high number of human and animal tumors as a measure of angiogenesis. The method is carried out counting the outline of blood vessel wall, labelled by immunohistochemistry for endothelial cell markers and obtaining the number of micro-vessels per square millimiters. Before performing the count, the slide is scanned at low power to identify "hot spots" with the highest blood vessel density. Neovascularization is distinguished if single, micro-vessels with a lumen are identified. Although apparently easy to perform, the method suffers the lack of standardization which makes the technique poorly reproducible and non-comparable across studies. In fact, there is no consensus as to what antibody should be used to label endothelial cells, what size is the area of observation and what criteria define a micro-vessel. Moreover, a degree of subjectivity is expected when selecting the hotspots. To overcome this issue, the Chalkley method has been proposed[7], which however, has not gained popularity amongst pathologists. The method is also called point-of-overlap morphometry and requires the use of a special attachment (the Chalkley graticule) to the eyepiece of the microscope. The total number of intersections of vessels with the

grid lines is counted. Unfortunately, to date there is no standard, objective and repeatable method to assess iMVD in tumors.
A number of studies have been published in veterinary medicine that evaluate IMVD in dogs and cats. These include, but are not limited to, mammary tumors, prostatic carcinomas, seminomas, squamous cell carcinomas, soft tissue sarcomas, mast cell tumors amongst others. Results of these studies indicate that increased iMVD is commonly associated with malignancy, vascular invasion, metastasis and overall survival.

**CD34** is also used to evaluate blood vessels, however, the antibody also labels hematopoietic stem cells therefore its expression is not exclusive for ECs. **CD105** (endoglin) is commonly used in human medicine to evaluate angiogenesis, but its expression has not been assessed for immunohistochemical use. In a recent study, the authors evaluated endoglin expression by RT-PCR, in different canine tumors, and found that endoglin mRNA expression correlated with VEGFR-2 and with iMVD, suggesting that endoglin has a potential for evaluation of tumor angiogenesis.[6] Claudin5 and thrombomodulin are two additional markers that are routinely applied to evaluate angiogenesis in tumors. However, these markers have not been tested in dogs and cats for immunohistochemical use. CD144 (Vascular Endothelial-Cadherin) is expressed by endothelial cells. It is an endothelial-specific cell-cell adhesion protein of the adherent junction complex, therefore its expression correlates with the stability of the junction and with vascular permeability. CD144 is also not routinely used in veterinary medicine.

Within the different types of angiogenesis mechanism, **vasculogenic mimicry** has been extensively evaluated in human tumors as well as in animals. Vasculogenic mimicry, which is the ability of neoplastic cells to form vessels without endothelial cells, can be assessed with double labelling with PAS (for basement membrane) and ECs markers (CD31 or vWF). Normal vessels are PAS+ and CD31+ while neoplastic cells mimicking vessels are PAS+/CD31-.[12] Whether this is a definitive proof of VM remains to be determined. Studies in canine mammary inflammatory carcinoma have identified VM using immunohistochemistry for cytokeratins. Results of this study describe CK+ vessels containing intraluminal emboli.[2]
Besides the identification of certain cells or vascular features in tissue sections, immunohistochemistry can be used to assess the presence of growth factors and other signaling molecules. The presence, but also the amount and localization of growth factors can be evaluated. Growth factors and receptors routinely evaluated in tissue sections include the VEGF, PDGF, FGF and Ang/Tie signaling pathways. These have been evaluated in a number of canine and feline tumors and were found to be associated with angiogenesis and malignancy.[1,10] Double immunolabelling for both growth factors and their receptors could be performed and can highlight autocrine and paracrine signals within tumors.
Similarly, *in situ* hybridization is a less used, and more expensive technique that has great potential to evaluate the amount of RNA to correlate with protein expression. Very few studies in dogs and cats have used this technique to evaluate angiogenesis in cancer.

Lastly, lymphatic vessels can be identified and evaluated in tissue sections with immunohistochemistry. Markers include **VEGF-C**, **VEGF-D** and **VEGFR-3** which label lymphatic endothelial cells.[11] Two recently introduced markers for LECs in dogs and

cats include **LYVE-1** and **Prox-1**. The performance of these two antibodies has been evaluated in canine mammary tumors, and Prox-1 appears to have achieved better results. These markers can be used to identify ECs within tumors. In addition, they could be applied to increase the sensitivity in identifying tumor emboli in lymphatic vessels, which carries well known difficulties.

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# ORAL PRESENTATIONS

# Canine Multiple Myeloma: a review on diagnosis, staging, treatment and prognosis with evaluation of novel data

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## Introduction

Publications regarding canine multiple myeloma (MM) are often older and limited to smaller case series. Hence, there is a lack of investigations into newer diagnostics, staging and prognosticators that may improve case management. The objective of this study was to evaluate use of current diagnostic criteria, staging, treatment and prognosticators in a large cohort of client-owned dogs with MM.

## Materials and methods

This was a retrospective, descriptive study assessing criteria for diagnosis, staging, treatment and prognostic factors in dogs with MM. Statistical analysis including descriptive and multivariable analysis was performed using Stata 15.

## Results

Ninety-one dogs were included. Lethargy (n=45, 49.4%), polyuria/polydipsia (n=32, 35.2%) and anorexia (n =29, 31.9%) were the most common clinical signs. Postulated ancillary criteria for diagnosis of MM including cytology, hypercalcemia, cytopenia and gammopathy were frequently used to obtain diagnosis. Melphalan/prednisolone (pulse dose or continuous) was the most common first-line protocol used (n=72), with no survival difference documented between the two treatment regimens. Median survival time (MST) for all dogs was 403 days (95% CI 250-497). Anorexia, organomegaly, polyuria/polydipsia, increased ALP, hypercalcaemia and elevated creatinine were negative prognostic factors. Anaemia and chemotherapy treatment were positive prognostic factors. Anorexia (p=0.019), organomegaly (p=0.023), anaemia (p=0.030) and chemotherapy (p = 0.003) remained significant in multivariable analysis.

## Conclusions

Clinical presentation, treatment protocol and MST were similar to previously reported for MM. Anorexia and organomegaly were newly identified negative prognosticators. The frequent use of ancillary criteria confirm the need for a consensus statement on diagnosis of MM in veterinary oncology.

# Chemotherapy improves survival in dogs with mesothelioma: a case-control retrospective study on 37 cases

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## Introduction

Mesothelioma is a rare cancer in dogs for which chemotherapy is often suggested despite no demonstration of its efficacy. The aim of the study was to evaluate the impact of chemotherapy on survival of dogs with mesothelioma. Based on our observations, we hypothesized that chemotherapy would be associated with a longer survival.

## Materials and methods

A restrospective multicentric study was carried out. Dogs diagnosed with pericardial, pleural, peritoneal or multicavitary mesothelioma (based on histopathology or cytopathology and evocative clinical evolution) between January 2004 and 2020 were included. Complete clinical follow-up was also required for inclusion. Group 1 comprised dogs that received chemotherapy and group 2 those that did not. Homogeneity between groups was assessed using Fisher's exact test or Mann-Whitney U test depending on the distribution of the variable. Survival analyses were performed using Kaplan-Meier method with log-rank tests.

## Results

37 dogs were included, 24 of which received chemotherapy. 17 dogs in group 1 and 2 dogs in group 2 also underwent surgery. There was no statistical difference between groups regarding sex, age, duration of clinical signs, number and location of effusions at diagnosis. Surgery had no significant influence on survival (p=0.87). Dogs from group 1 survived significantly longer than dogs from group 2 (MST: 366 vs 74 days; p

## Conclusions

Chemotherapy seems to improve outcome of canine mesothelioma and should be considered as part of the treatment.

# Adjuvant vinorelbine in the management of pulmonary carcinoma in dogs

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## Introduction

The adjunctive role of chemotherapy for primary pulmonary carcinoma (PC) has not been elucidated. Vinorelbine holds promise as an adjuvant chemotherapy drug for canine PC due to its high lung concentrating ability. The aim of this study was to establish outcome for dogs with PC managed with surgery and vinorelbine (Sx/Vb), surgery alone (Sx), or no treatment (NT).

## Materials and methods

Dogs with PC presenting to a single institution from 2012 to 2019 were included if they had a cytological/histological diagnosis and treatment with Sx/Vb, Sx or NT. Time to progression (TTP), and survival time (ST) were evaluated for each dog.

## Results

Forty-six dogs met the inclusion criteria; Sixteen, 10 and 20 dogs in the Sx/Vb, Sx and NT group respectively. The median follow-up was 407 days (407-1225). The median TTP (MTP) for Sx/Vb, Sx and NT were 131.5 (54-512), 130.5 (9-1225) and 17 days (0-771), respectively. An improved MTP was found for Sx versus NT (P= 0.044) and Sx/Vb versus NT (P=0.016). The median ST (MST) for Sx/Vb, Sx and NT were 195 (range 75-1386), 150.5 (9-1225) and 35 days (0-771), respectively. A superior MST was found for Sx versus NT (P=0.038), and Sx/Vb versus NT (P=0.0001).

## Conclusions

An increase in MTP and MST was noted for dogs that received Sx or Sx/Vb versus NT. No significant difference in outcome was detected between Sx and Sx/Vb. Poor median outcomes were found despite adjunctive chemotherapy, however large prospective studies are required to further investigate the role of adjunctive chemotherapy in dogs with PC.

# Efficacy and tolerability of zoledronate in the treatment of dogs with hypercalcemia of malignancy or tumour associated bone pain

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## Introduction

Zoledronate is a bisphosphonate frequently used for the treatment of hypercalcaemia of malignancy (HM) and tumour associated bone pain (TBP) in dogs, however information regarding its efficacy and tolerability in veterinary medicine is lacking. The aim of this retrospective study was to report the tolerability and efficacy of zoledronate in the palliative treatment of cancer bearing dogs.

## Materials and methods

Case records of 106 zoledronate infusions (0.062mg/kg – 0.25mg/kg) administered to 39 dogs (24 with TBP and 15 with HM) were reviewed. Efficacy was assessed by subjective improvement of clinical signs and resolution of hypercalcaemia (HM). Tolerability was assessed by absence of post-zoledronate hypocalcaemia or other adverse events (AE) as defined by VCOG-AE criteria.

## Results

In 84% of dogs, a clinical benefit was noted following treatment with zoledronate, with improvement or resolution of clinical signs associated with either HM or TBP. In hypercalcaemic patients, ionized calcium (iCa) decreased rapidly within 7 days (median iCa, before = 2.17mmol/L, after = 1.28mmol/L). In 81% of zoledronate infusions, no AE were reported. The majority of AE could potentially be directly attributed to concurrent chemotherapy or the underlying neoplastic disease. There was one episode of symptomatic hypocalcaemia in a hypercalcaemic patient treated with zoledronate and L-asparaginase for refractory T-cell lymphoma, but none in dogs treated for TBP that were normocalcaemic or hypocalcaemic before treatment. Baseline creatinine did not increase significantly following zoledronate treatment.

## Conclusions

Zoledronate is an effective palliative treatment for dogs with HM or TBP. It is well-tolerated with few recorded AE and rarely causes clinical hypocalcaemia.

# Evaluation of intravesical administration of carboplatin in association with nonsteroidal anti-inflammatory drugs in dogs with bladder urothelial carcinoma- retrospective study

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## Introduction

Currently, UC corresponds to 1 to 2% of all types of neoplasia affecting dogs. Despite this low incidence, at the time of diagnosis, 20 to 37% of neoplasias already presented metastases, 58% had a diffuse character and 60% were in the trigone. Therefore, chemotherapy is one of the best therapeutic options.
In Human Medicine, intravesical chemotherapy is already quite common. However, in Veterinary Medicine there is little research that studies this therapy. Thus, the objective of this study was to evaluate the tumoral response of UC to intravesical administration of carboplatin and nonsteroidal anti-inflammatory drugs therapy.

## Materials and methods

The retrospective study included ten dogs with confirmed UC either by cytology or histopathology. The intravesical administration of carboplatin (300mg/m2) was performed every three weeks, concomitantly with the daily administration of NSAID. All dogs had a baseline abdominal ultrasound which was repeated every 4-6 weeks to monitor the disease. Tumor response was assessed according to cRECIST guidelines. Toxicoses were graded according to VCOG criteria.

## Results

It was possible to observe a complete response in four of the ten dogs included in the study and a partial response in one. The median survival time was not calculated since 60% of the animals were still alive by the end of the study. However, the survival time ranged from 130 to 998 days.

## Conclusions

With a response rate of 50%, this protocol is a viable alternative for the treatment of dogs with UC. Further research with larger samples and possible adjustments in the dosage should be performed.

# Host and Viral Transcriptomic Features of Equine Sarcoids

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## Introduction

Sarcoids are common tumours of dermal fibroblasts caused by Bovine Papillomavirus (BPV) -1/2, affecting equids. This study characterises gene expression profiles associated with these tumours to better understand pathogenesis and develop biomarkers.

## Materials and methods

Normal equine fibroblast, BPV-1 infected fibroblast and sarcoid cell lines were cultured in vitro. Additionally, samples of normal skin and sarcoid tissue from horses and donkeys were collected. Messenger RNA (mRNA) and microRNA (miRNA) were extracted and Illumina sequencing performed. Reads were mapped to viral and equine genomes and Principle Component Analysis (PCA), differential expression and pathway analysis were performed to provide an overview of transcriptional differences among groups.

## Results

Different cell lines clustered separately based on host miRNA and mRNA profiles on PCA. Normal and sarcoid tissue could also be distinguished based on these profiles. A higher proportion of sarcoids contained BPV-1 than BPV-2, and viral gene expression was very similar in BPV-transformed fibroblasts and sarcoid tissue. Sarcoids with the highest viral load provided the most distinctive host gene expression profiles. Donkeys and horses showed similar results, with pathways associated with cancer, dermatological diseases and fibrosis showing significant enrichment. Several genes known to be up- or down-regulated in multiple human cancers, or that have been previously associated with sarcoids, were dysregulated ($Padj<0.01$).

## Conclusions

BPV gene expression was invariant among the sarcoids so viral load and host factors may be the primary drivers of sarcoid phenotype. The in vitro line provides a helpful model. Sarcoids in donkeys and horses were transcriptionally indistinguishable; common mRNA and miRNA biomarkers may be identified.

# The use of cannabinoids for canine medical conditions among Danish dog owners

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## Introduction

In recent years there has been increasing interest in use of medical cannabinoids. In Denmark there are no registered veterinary medical cannabinoid products, but the impression is that cannabinoids are used frequently in dogs. The aim was to explore use of cannabinoids in Danish dogs including indications for and owner perceived effects of the treatment.

## Materials and methods

The data collection was performed via an anonymous online survey distributed via Copenhagen University Hospital for Companion Animals' Facebook page. Respondents were asked about demographics, dog breed category, cannabinoid use, indication/s and their perception of effect.

## Results

Of 2002 individual respondents 38 % (n=753) reported having used cannabinoids. Most commonly used was cannabidiol (CBD) drops/oil (92,6 %, n=696). The major indications were pain (50 %, n=377), behavioral-issues (19 %, n=149) and allergy (16 %, n=122). Nine percent (n=70) used it as anti-neoplastic treatment. Seventy-seven percent (n=580) of respondents perceived good or very good effect. When used for pain good effect was perceived in 83 % (n=312) while 72 % and 73 % respectively reported good effect when used for behavioral-issues and allergy. Comparisons revealed a significant difference between the mean score of the effect in the pain-group and the allergy-group ($p=.023$) but not between other indications ($p>.05$).

## Conclusions

This study supports that even in areas where veterinary medical products are not accessible, there is a large segment of dogs treated with cannabinoids for medical purposes. The owner perceived effect is encouraging and supports the need for more evidence-based knowledge within the veterinary field of medical cannabinoids.

# Fluorescence-guided surgery in dogs with superficial tumors using a novel integrin-targeting near-infrared fluorescent contrast agent

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## Introduction

Recurrence due to incomplete tumor resection is one of the major causes of death in human and canine cancer patients. Fluorescence-guided surgery may facilitate complete resection of solid tumors through intraoperative visualization of tumor margins. Preliminary safety and imaging efficacy of a novel AvB3-integrin targeting near-infrared fluorescent (NIRF) agent (DA364) was evaluated during surgical resection of solid tumors in dogs.

## Materials and methods

DA364 was administered intravenously to dogs with solid tumors. Intraoperative NIRF imaging was performed in situ and on excised specimens to evaluate fluorescence intensities of tumor and adjacent (healthy) tissues. After standard-of-care tumor resection, the wound bed was imaged again, and additional tissue was excised if residual fluorescence was detected.

## Results

Thirty-two superficial solid tumors were removed in 24 client-owned dogs after administration of DA364. No side effects were observed. The median in vivo tumor-to-background ratio for mammary tumors, mast cell tumors and sarcomas was 1.8 (range 1.2-3.9), 2.2 (range 1.0-5.6), and 4.2 (range 2.0-4.3), respectively. Fluorescence was detected in 4 wound beds that contained residual disease and in 12 clean wound beds, confirmed by histopathology. Ex vivo, the median tumor-to-margin ratio for mammary tumors, mast cell tumors and sarcomas was 3.4 (range 1.6-6.4), 2.6 (range 2.0-12.2), and 3.3 (range 1.2-5.4), respectively. Resected metastatic lymph nodes showed greater contrast than tumor-free nodes.

## Conclusions

The probe can detect different tumor types and pinpoint residual disease in the wound bed, but can suffer some limitations due to nonspecific detection of false positives. Larger clinical trials are warranted for further evaluation of the contrast agent.

# Treatment of feline nasal planum squamous cell carcinoma (fNP-SCC) using electrochemotherapy (ECT) alone

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## Introduction

ECT, which combines the use of cytotoxic drugs and reversible electroporation is considered a treatment option for fNP-SCC. The feasibility and efficiency of ECT in the treatment of a multicentric retrospective case-series of fNP-SCC were evaluated.

## Materials and methods

Twenty-one cats with fNP-SCC without evidence of metastasis, treated with neoadjuvant ECT (bleomycin IV, 15000 UI/m2), administered 8-10 minutes before pulse delivery (8 pulses, 100 µs/pulse, 1200 V/cm) were enrolled. Relationships between age, sex, tumour size, number of ECT treatment, type of presentation (first/recurrence), response to treatment (CR/PR/SD/PD), local toxicity and survival time (ST) were evaluated using Mann-Whitney test, Fisher's exact test, and Kaplan-Meier curve analysis. Six cats, still alive, were censored from survival analysis.

## Results

Median (range) of age, tumour size, follow-up (alive) and ST (dead) were as follows respectively: 11 years (5-15), 10 mm (3-60), 193 days (29-2,929), and 268 days (30-2,929). Two cats were treated for recurrence of previously excised fNP-SCC and only three cats received two ECTs rather than one. Response rate was 90.5% (15 CR, 4 PR). Two cats showed SD surviving for 160 and 284 days after ECT. Local treatment toxicity was mild (16/21). Median tumour size was significantly smaller in cats with CR compared to those with PR/SD (7 vs. 16 mm, p= 0.008). No ECT variables were associated with local toxicity. Cats with CR lived longer than cats with PR/SD (394 vs. 193 days, p= 0.022).

## Conclusions

Smaller fNP-SCC showed a better response to ECT and CR was associated with longer survival time.

# New potential tyrosine kinase inhibitors (TKIs) for treatment of canine mast cell tumors (MCTs)

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## Introduction

Advanced canine mast cell tumors (MCTs) are defined by uncontrolled growth of neoplastic mast cells (MCs) and accumulation of these cells and their inflammatory mediators in one or more organs. Despite the approval of two KIT tyrosine kinase inhibitors (TKIs), namely masitinib and toceranib, relapses are seen frequently. Therefore, more effective anti-neoplastic drugs are needed to treat recurrent or metastatic MCTs.

## Materials and methods

We tested the effects of three novel TKIs, namely avapritinib (BLU-812), ripretinib (DCC-2618), and nintedanib (BIBF-1120) on two established canine MC lines, C2 and NI-1 and on primary MCs obtained from a MCT patient (n=1). The TKIs masitinib, toceranib and midostaurin were used as control agents.

## Results

All TKIs were found to inhibit proliferation of both MC lines with IC50 values ranging between 0.01 and 0.25 µM (rank order of potency: toceranib>ripretinib>nintedanib>masitinib>avapritinib>midostaurin). Moreover, all TKIs decreased survival in C2 cells with ED50 values ranging between 0.05 and 1 µM (toceranib>nintedanib>ripretinib>masitinib>midostaurin>avapritinib), whereas in NI-1 cells ED50 values ranged between 0.5 and 10 µM (toceranib>ripretinib>nintedanib>midostaurin>masitinib>avapritinib). Finally, the TKIs were found to reduce IgE-dependent histamine release in NI-1 cells and in primary MCT cells (nintedanib>midostaurin>ripretinib>avapritinib>toceranib >masitinib).

## Conclusions

In summary, the TKIs avapritinib, ripretinib and nintedanib suppress growth, survival and mediator release in neoplastic canine MCs. The effects of these new drugs were comparable to the effects of masitinib, toceranib and midostaurin. Whether these TKIs are similarly effective in vivo when compared to masitinib or toceranib remains to be elucidated in clinical studies.

# Benefit of hepatic and splenic cytology in dogs with cutaneous mast cell tumors in the presence and absence of negative prognostic factors

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## Introduction

In dogs with cutaneous mast cell tumors (cMCTs), staging, including hepatic/splenic fine needle aspiration (FNA), is generally recommended based on histological tumor grade and the presence of other negative prognostic factors (NPFs). However, the rate of organ metastasis without NPFs has not been directly investigated. The purpose of this study was to evaluate the benefit of hepatic/splenic cytology in cMCTs with or without NPFs.

## Materials and methods

Dogs with cMCTs that underwent staging, including splenic/hepatic FNA, between 2007 and 2018 were identified. Patients were grouped based on the presence or absence of histological (Patnaik grade III/Kiupel high-grade) and clinical (rapid growth, recurrence, high-risk location) tumor NPFs. A minimum of one-year follow-up was required. Kaplan-Meier method was used to compare survival times (log-rank test $p<0.05$).

## Results

Hundred and four dogs were included, with 48 (46%) cMCTs having and 56 (54%) lacking NPFs. There was a significant difference (p

## Conclusions

The results indicate that in canine cMCTs, in the absence of NPFs extensive staging, including splenic/hepatic FNA, is not beneficial. Moreover, using the described NPFs, a subset of cMCTs prone to organ metastasis can be successfully identified.

# Diagnostic performances of lymph node cytopathology in predicting lymphoma and its who histotype in dogs

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## Introduction

The World Health Organization (WHO) classification of canine nodal lymphomas is mainly based on histopathology and immunohistochemistry (HIS+IHC). The invasive sampling procedure and the reporting time represent a deterrent to the execution of this test, and many clinicians rely on cytopathology only. The aim of the present study was to assess the diagnostic performances of cytopathology in identifying: 1) canine nodal lymphoma, 2) immunophenotype and grade, 3) WHO histotype.

## Materials and methods

161 cytopathological glass slides from enlarged peripheral lymph nodes were blindly submitted to 6 examiners. Final HIS+IHC diagnoses were obtained. The examiners indicated: 1) lymphoma vs non-lymphoma; 2) supposed grade and immunophenotype; 3) supposed WHO histotype.

## Results

Cytopathology was accurate and precise in identifying lymphoma samples (sensitivity = 93.1%, specificity = 89.5%, Kappa inter-observer agreement = 0.79). Agreement was only moderate in identifying grade and phenotype (k= 0.41) and WHO subtype (k=0.41). Only 48.6% evaluations agreed with final diagnosis according to grade and phenotype and 38.7% according to the WHO subtype.

## Conclusions

Cytopathology alone may accurately diagnose lymphoma but its accuracy drops when further characterization is needed. When B-cell lymphoma is suspected cytopathologically, the phenotype is likely to be confirmed via HIS+IHC. Caution should be made in reporting a high-grade lymphoma by cytopathology, particularly if B-cell phenotype is suspected. Histopathology and IHC are mandatory for a definitive diagnosis of the lymphoma WHO histotype. The role of further diagnostic techniques should be addressed in future studies.

# Interest of whole-body immuno-SPECT-CT for canine diffuse large B-cell lymphoma (DLBCL) staging before radioimmunotherapy (RIT) using an anti-CD22 monoclonal antibody

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## Introduction

Anti-CD22 immuno-SPECT-CT (three-dimensional scintigraphy) has been shown to be sensitive for whole-body staging in human patients with diffuse large B-cell lymphoma (DLBCL). Development of this diagnostic option in veterinary medicine should offer an alternative to time-consuming conventional staging. We have evaluated this imaging method in dogs with DLBCL.

## Materials and methods

Five dogs with CD22-overexpressing progressing DLBCL received intravenous injection of the murine monoclonal anti-CD22 antibody DOTA-10C6 radiolabeled with indium-111. Four successive SPECT-CT acquisitions were performed under anesthesia to confirm staging, validate tumor targeting. In parallel, conventional staging was performed by thoracic x-ray, abdominal ultrasound, CT examination, liver and spleen cytology and bone marrow aspiration. Same work was done in nine healthy dogs to distinguish tumor from physiological uptake. The concentration of antibodies present in the parenchyma of the organs of interest was calculated using the quantification of SPECT images for both healthy and sick dogs.

## Results

Signal due to antibody concentration into infiltrated lymph nodes made them visually recognizable on SPECT-CT images. Using the quantification of SPECT acquisitions, we have revealed bone marrow infiltration in two more dogs compared to bone marrow aspiration. Compared to abdominal ultrasound, spleen infiltration was diagnosed in one more dog and lymph node infiltration in three more dogs using SPECT image quantification. All scintigraphy findings were confirmed by cytology, for organs.

## Conclusions

SPECT-CT acquisition performed 48 hours after 111In-DOTA-10C6 injection allowed a successful whole-body staging compared to gold standard and bone marrow cytology and conventional imaging techniques.

# WNT-genes and VEGFR -1/2 are overexpressed in T-Cell lymphomas, but do not predict treatment response

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## Introduction

Although canine lymphoma is commonly treated, predicting treatment response and prognosis remains challenging. Tumour characteristics related to angiogenesis, cell proliferation and apoptosis are prognostic indicators in many cancer types. The aim of this study was to assess whether qPCR analysis using a minimally invasive (aspiration) tumour biopsy would allow for the measurement of several of these genes and might prove of prognostic value.

## Materials and methods

Biopsies were collected from 57 treatment-naive dogs with multicentric lymphoma at diagnosis (n=57), at relapse (n=34) or following treatment failure (n=22). All samples were stored at -80°C till analysis and qPCR-analysis was performed in duplicate. In total 27 genes related to immunophenotype, angiogenesis, cell proliferation and apoptosis were measured. Fold-change s(Fc) were calculated using the 2-??Ct-method. Results were tested for normality and based on outcome tested with appropriate parametric or non-parametric tests. Significance was set at $P < 0.05$.

## Results

CD3 and CD19 mRNA expression differentiated between B- (n=44) and T-cell (n=13) lymphomas. T-cell lymphomas showed higher expression of multiple Wnt-pathway genes (FC: 2.5 - 76) and VEGFR-1 and -2 (FC: 2.8 - 3.3). There were no significant differences between B- and T-cell lymphomas in the expression of genes related to proliferation and apoptosis or between drug-sensitive and drug-resistant samples.

## Conclusions

qPCR can be used to differentiate between B- and T-cell lymphomas. Genes related to Wnt-pathway and angiogenesis, but not proliferation or apoptosis, showed higher expression in T-cell lymphomas. None of the genes other than immunophenotype were related to treatment response.

# Retrospective study of T-cell leukaemia (large granular lymphocytes variant) in dogs associated with suspected immune-mediated cytopenias in absence of peripheral lymphocytosis

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## Introduction

Large granular lymphocyte (LGL) leukaemia is characterised by clonal expansion of cytotoxic T or natural killer lymphocytes. In humans, it is often associated with autoimmune disorders, including immune-mediated cytopenias. In dogs, only two cases have been described to date. LGL leukaemia typically affects middle-aged to older dogs and is characterised by circulating lymphocytosis without significant cytopenias. The aim of this study was to describe clinical characteristics, treatment and outcome of dogs with LGL leukaemia presenting with suspected immune-mediated cytopaenia.

## Materials and methods

Inclusion criteria were a diagnosis of LGL leukaemia with associated single or multiple cytopaenias on presentation.

## Results

Six dogs were included in the study. The median age was 4 years (2-6 years). The most common presenting clinical signs were fever and lethargy. All dogs had severe neutropenia (median neutrophil count 0.07x109/L), three had concurrent thrombocytopenia (median platelet count 66x109/L), and one anaemia (HCT 0.32 L/L). None had a circulating lymphocytosis. In all dogs, bone marrow cytology revealed the infiltration of granular lymphoid T-cells; clonality was confirmed in 3 dogs and flow cytometry was performed in two cases, identifying CD3+, CD8+ neoplastic cells. All patients were treated with systemic chemotherapy. Two dogs were euthanised 133 and 322 days after diagnosis due to progressive disease; one patient was lost to follow-up after 357 days. The remaining three patients were alive, at a median follow-up of 350 days (217-721days).

## Conclusions

As in humans, a subset of LGL leukaemia in dogs is associated with immune-mediated cytopaenia. This has a unique clinical presentation and younger dogs are affected.

# Prognosis of feline gastrointestinal small cell transmural lymphoma

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## Introduction

Feline gastrointestinal lymphoma is classified as large cell, small cell or large granular lymphocyte (LGL) types depending on the cellular morphology and size, and as mucosal lymphoma (lymphocytic infiltrate confined to epithelium and lamina propia) or transmural lymphoma (when infiltrate extends into the submucosa and muscularis propria). The prognosis for large cell is worse, with a MST of 1.5 months compared to 28 months for small cell lymphoma. Similarly, prognosis of transmural lymphoma is shorter (1.5 months) that mucosal (29 months) in a study with 120 cats (Moore PF, Vet Pathol 2011). However, in this study most of the transmural cases were large cell lymphomas. Within the cases that had survival data available, 9 out of 13 transmural lymphomas were large cell lymphomas with most of these (8/9) being LGL. Our hypothesis is that small cell transmural lymphomas have a better prognosis and that the extension to muscularis is not a bad prognostic indicator by itself.

## Materials and methods

We retrospectively evaluated all the cases at two institutions that fulfilled the following criteria: 1) surgical full thickness biopsy, 2) chemotherapy treatment, 3) follow up of at least five months or until death and 4) no concomitant diseases that could impact the prognosis.

## Results

14 cases were included in the survival analysis. Median survival time was 690 days (23 months), with 1 and 2 year survival rates of 78% and 48%% respectively.

## Conclusions

In conclusion, feline gastrointestinal small cell transmural lymphoma has a similar prognosis to the small cell mucosal type.

# Differentiation between cortical and medullary origins in canine adrenal tumors using contrast-enhanced ultrasound vs plasma catecholamines vs urinary metanephrines

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## Introduction

The purpose of this study was to compare contrast-enhanced ultrasound (CEUS), plasma catecholamine fraction and urinary metanephrine fraction for the differentiation of origins in canine adrenal tumors.

## Materials and methods

Forty-three client-owned dogs were included in this study. Plasma concentrations of catecholamines (adrenaline, noradrenaline and dopamine) and urinary concentrations of fractionated metanephrines (metanephrine and normetanephrine) and creatinine were preoperatively measured. In addition, CEUS was performed, and time intensity curve (TIC) was derived from the region of interest placed within the mass for the calculation of 5 parameters.
All dogs underwent surgical treatment, so definitive diagnosis was histopathologically made. All dogs were divided into adrenocortical (AC) group (adrenocortical adenoma and adenocarcinoma) and adrenomedullary pheochromocytoma (PHEO) group. For differentiating between AC and PHEO groups, diagnostic accuracy of plasma catecholamines, urinary metanephrines, and the parameters of CEUS was evaluated.

## Results

Of 43 dogs, 6, 11 and 26 were histopathologically diagnosed as adrenocortical adenoma, adrenocoritical adenocarcinoma, and PHEO, respectively. For the detection of PHEO in all dogs, the sensitivity and specificity of CEUS parameters were 38-69% and 94%, respectively. The sensitivity and specificity of plasma catecholamines were 69-92% and 47-59%, respectively. In 12 dogs that the urinary metanephrines were measured, the sensitivity and specificity of urinary metanephrines were 83.3% and 66.7%, respectively.

## Conclusions

This study suggested that plasma catecholamines and urinary metanephrines had high sensitivity whereas CUES had high specificity. The combination of those examinations might improve the accuracy for the differential diagnosis of adrenal tumor origin.

# Vitamin D status in female dogs with mammary tumours

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## Introduction

Mammary tumours are the most common neoplasm in female dogs. Vitamin D deficiency is common in breast cancer patients and some evidence suggests that low vitamin D status enhances the risk for disease development or progression. Decreased blood 25(OH) D3 concentrations also have been reported in dogs with different neoplasm. Our objective is to study vitamin D status in female dogs with mammary tumours where there is no information.

## Materials and methods

We collected blood from 80 female dogs with mammary cancer before the surgery and 40 aged matched healthy female control dogs. Plasma ionized and total calcium, phosphate, magnesium, parathyroid hormone, 1,25 (OH)2 D3 and 25 (OH) D3 levels were measured in all the animals. Data are presentea as media +/- standard error.

## Results

We found no significant differences between groups in any of studied parameters. Plasma 25(OH) D3 levels were very similar in control (152.6 +/-14.2 ng/ml) and tumour (161.7+/- 8.7 ng/ml) groups. Also, when we compare plasma 25 (OH) D3 concentrations in dogs with malignant (148.6+/-6.4 ng/ml) vs benign (168.0+/-18.2 ng/ml) tumours, no significant differences were found (p=0.322). Age and ionized calcium were positive correlated with histologic grade and RECIST scale of the tumours.

## Conclusions

The status of vitamin D and the main parameters of mineral metabolism in female dogs with mammary tumours are not altered. Based on these data, 25-hydroxyvitamin D insufficiency might not be important in the pathogenesis of canine mammary tumours.

# Human TNBC marker gamma Klotho is upregulated in canine mammary carcinomas: a preliminary study

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## Introduction

Breast cancer is the most prevalent cancer in women and intact female dogs. In humans, triple-negative breast cancer (TNBC) remains a challenging problem due to aggressive clinical course and a lack of targeted therapies. γKlotho was recently discovered as a novel marker of a subset of TNBCs, it was overexpressed in more than 60% of patients with TNBCs and correlated with poorer disease progression. It was shown that γKlotho confers protection against oxidative stress and might be a potential marker for patients that would benefit from oxidative therapy. Given that canine mammary carcinoma (MC) often resembles human TNBCs, we hypothesized that γKlotho could be also relevant to dogs.

## Materials and methods

We have analyzed the expression of γKlotho in the MC obtained from female dogs of different ages (6.5– 11.5 years) and breeds, which underwent surgery. After surgical removal, tissue was immediately frozen in the liquid nitrogen and stored at -80 until further processed. Before RNA extraction, tissue was powdered and RNA was extracted and converted to cDNA for gene expression analysis by quantitative PCR using Sybr Green technology. The expression was normalized to 18s rRNA and compared to the expression in adjacent benign mammary gland tissue.

## Results

γKlotho is significantly upregulated in MC compared to the normal mammary gland, suggesting a role of γKlotho in the oncogenesis of the human TNBC and MC.

## Conclusions

These results warrant further investigation into γKlotho as a potential prognostic biomarker and a novel therapeutic target for human and canine breast cancer patients.

# Prognostic value of c-Myc overexpression in feline invasive mammary carcinomas

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## Introduction

c-Myc, a transcription factor with pleiotropic effects including cell proliferation, is overexpressed in some breast cancers and associated with shorter disease-free survival. Here, we investigated c-Myc expression and prognostic value in feline invasive mammary carcinomas (FMCs).

## Materials and methods

We retrospectively analyzed 180 FMCs from 180 female cats treated by surgery alone. C-Myc (clone Y69), Estrogen Receptor (ER), Progesterone Receptor (PR), Androgen Receptor (AR), Ki-67, HER2 and cytokeratin-14 (CK14) expressions were determined by automated immunohistochemistry. A percentage of c-Myc–positive neoplastic cells (c-Myc index) higher than 57% defined c-Myc overexpression.

## Results

c-Myc overexpression was found in 38% (69/180) of the FMCs, and was significantly associated with a higher mitotic index (p=0.02), higher Ki-67 index (p=0.002), AR (p=0.01) and CK14 (p=0.02), but not tumor stage, histological grade, ER and PR. The prognostic value of c-Myc overexpression was significant only in smaller FMCs (pathologic tumor size pT1, < 20 mm in diameter, N=85), in which c-Myc overexpression was associated with a 2-fold increased risk of cancer-related death (Hazard Ratio 2.34, 95% confidence interval 1.33–4.15, p=0.004), independently of lymphovascular invasion (HR=2.54), ER index (HR=1.04) and AR positivity (HR=0.36).

## Conclusions

c-Myc overexpression is a strong unfavorable prognostic factor in FMCs, but only in smaller (pT1) ones, suggesting that, with cancer progression, other events than c-Myc overexpression become predominant in cancer aggressiveness. Defining prognostic factors of pT1 FMCs is important, as better medical management of cats will likely lead to earlier FMC diagnosis, and increasing proportions of pT1 than pT2 FMCs.

# Downregulation of CD146 sensitizes radio-resistant canine oral melanoma cell lines to irradiation

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## Introduction

Canine oral malignant melanoma (COMM) is a very aggressive tumour disease. High surface expression of cell adhesion protein CD146 is reported to promote tumour progression and metastasis. Current treatment strategies often fail to improve median survival times in COMM patients.

## Materials and methods

We assessed the effect of CD146 downregulation on radio-sensitivity in CD146-positive primary COMM cell lines. To this aim, cells were transfected with anti-CD146 or unspecific siRNA (control), irradiated with 6 Gy or left untreated, and subjected to proliferation assay.

## Results

Irradiation of COMM cells explanted from primary tumours resulted in hypo-proliferation independent of CD146-targeted siRNA treatment. In contrast, irradiation of cells derived from lymph node metastases had no impact on proliferation. Importantly, radio-sensitivity of these cells could be fully restored by siRNA-mediated CD146 downregulation, as revealed by significant inhibition of proliferation following siRNA transfection and irradiation.

## Conclusions

Obtained data suggest that resistance of COMM cells to radiotherapy is co-induced by CD146, and that this effect can be reversed by CD146 blockade. Provided that this new finding can be confirmed in vivo, inhibition of CD146 prior to radiotherapy may represent an interesting therapeutic approach in COMM patients.

# A chimeric Human/Dog CSPG4 DNA vaccine reveals potential therapeutic effects against malignant melanoma

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## Introduction

Canine oral malignant melanoma (COMM) accounts for 30–40% of oral tumors, with 80% of dogs presenting metastatic disease. Standard therapies are mainly surgery and/or radiotherapy while chemotherapy has very little benefits; however once the tumor has metastasized, the 1-year survival is only 30%. Therefore, more effective treatments are needed. Being the chondroitin sulfate proteoglycan (CSPG)4 a promising immunotherapeutic target over-expressed in about 60% of COMM, we demonstrated the safety and the clinical relevance of a human (Hu)-CSPG4 DNA vaccine delivered by electroporation (electrovaccination) in dogs with stage II-III surgically resected CSPG4+ COMM. Nevertheless, some vaccinated dogs eventually died because of metastasis. Based on these results, we aimed at improving this immunotherapeutic approach and its possible translational power.

## Materials and methods

We generated a hybrid plasmid, derived in part from the Hu- and in part from the dog (Do)-CSPG4 sequences (HuDo-CSPG4). We tested the safety, immunogenicity and anti-tumor potential of HuDo-CSPG4 DNA electrovaccination in dogs with stage II-IV surgically resected CSPG4+ COMM and in a human setting in vitro.

## Results

Adjuvant HuDo-CSPG4 DNA electrovaccination in COMM patients is safe and induces high-affinity antibodies, endowed with anti-tumor potential, against both Hu- and Do-CSPG4. Clinically, the adjuvant HuDo-CSPG4 electrovaccination is effective in increasing the overall survival of vaccinated COMM patients as compared to controls. Moreover, data obtained in vitro with T cells from human donors suggest that HuDo-CSPG4 is potentially immunogenic.

## Conclusions

These results provide the rationale to propose HuDo-CSPG4 electrovaccination for the treatment of CSPG4+ COMM tumors, to be potentially translated also in a human setting.

# POSTER PRESENTATIONS

# Ablation of near infra-red stable transfected prostate cancer cell lines by C-CPE gold-nanoparticle mediated laser intervention

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## Introduction

Claudin (CLDN) genes are deregulated in prostate cancer. C-terminal domain of Clostridium perfringens enterotoxin (C-CPE) binds several CLDNs and thus recombinant C-CPE conjugated to gold nanoparticles (AuNPs) has been successfully used for in vitro cancer cells targeting. Inoculation of cancer cells is routinely used to generate in vivo models to understand the pathogenesis of prostate cancer and to evaluate novel therapeutic approaches. However, detailed characterization of cancer spreading and early tumour development and therapeutic response is often limited as conventional cell lines do not allow advanced deep tissue imaging. Herein, we established two CLDN expressing canine prostate carcinoma cell lines, stably expressing near infra-red (NIR) fluorescent proteins for future xenograft model studies.

## Materials and methods

Expression of NIR marker protein as well as CLDN3, -4 and -7 was confirmed by flow cytometry, qPCR and immunofluorescence. C-CPE binding to CLDNs was investigated through immunostaining and electron microscopy. Cancer cells ablation was demonstrated in an in vitro setting using a combination of gold nanoparticles mediated laser perforation (GNOME-LP) technique and C-CPE-AuNPs.

## Results

C-CPE binding to native and NIR transfected cancer cells verified the capability of C-CPE binding to specifically target CLDN receptors. Application of GNOME-LP reduces tumor cell viability to less than 10 % depending on cell line.

## Conclusions

The established cell lines and the verified proof of concept in vitro provide the basis for perspective in vivo studies. The introduced red fluorescence enables deep tissue imaging in living animals and therefore detailed characterization of tumor growth and subsequently possible tumor ablation through C-CPE-AuNPs treatment.

# Sensitivity of canine lymphoma/leukemia cells to estrogen receptor beta agonist

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## Introduction

Cellular signaling by estrogens is moderated via estrogen receptors ? (ER?) and ? (ER?). It was proven that selective ER? agonist inhibits proliferation of human and mouse lymphoma cells in vitro.This study aimed to determine the sensitivity of canine lymphoma/leukemia cells to selective ER? agonist and compare the results with estrogen receptors expression.

## Materials and methods

Cytotoxic effect of selective ER? agonist (Diarylpropionitrile, DPN) was checked against canine lymphoma/leukemia cell lines: GL-1 and CLB70. Cell metabolic activity was measured by MTT test, death induction was determined by propidium iodide staining after 72 and 96h. Expression of ER? and ? was examined by western blot in a broader panel of cell lines: GL-1, CLB70, CNK89, CLBL-1.

## Results

DPN cytotoxic effect on GL-1 and CLB70 cell lines was shown as a decrease in metabolic activity and cell death.The percentage of dead GL-1 cells was 43.0±8.0 and 76.80±5.09 and CLB70 cells was 54.2±3.8 and 85.7±4.1 after 72 and 96 h of incubation, respectively. Expression of the ER? receptor was demonstrated in all tested cell lines while only a weak expression of ER? was noticed.

## Conclusions

The study showed a cytotoxic effect of ER? agonist on canine lymphoma and leukemia cells. A strong expression of ER? in all cell lines with slight expression of ER?, may indicate a different mechanism of action of DPN in canine cells in comparison to human and mouse cells. DPN toxicity may not be associated with ER? stimulation or there may be a different role of specific types of estrogen receptors in canine cells.

# Thermographic analysis of dogs with soft tissue sarcomas subjected to synthetic phosphoethanolamine neoadjuvant therapy (Pho-S)

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## Introduction

Thermography is a physiological imaging technique that detects superficial temperature variations resulting from changes in cellular metabolism and blood flow. Tumors treated with synthetic phosphoethanolamine (Pho-S) demonstrated histologic confirmation of reduction in number of vessels and neovascularization.This study evaluated the temperature variation between the tumoral area (TA) and non-tumoral area (nTA) in 10 dogs with soft tissue sarcoma that received four weekly chemotherapy sessions with Pho-S (70 mg/kg/IV) as neoadjuvant therapy before oncologic surgery was performed.

## Materials and methods

Thermographic imaging of patients obtained with FLIR T650sc camera before treatment (T0) and before each Pho-S application (T1-4) was analyzed by FLIR R Research Software for temperature assessment. Difference in temperature (TA-nTA) was calculated and recorded prior to treatment administration (T0) and compared to the differences in temperatures obtained during subsequent treatment times (T1, T2, T3, and T4). Values lower than p

## Results

TA-nTA analysis elicited “decrease in tumor temperature when correlated to the body temperature” during neoadjuvant therapy (TA-nTA T1-4 < TA-nTA T0). Statistically significant difference (p=0.01416) was elicited between TA-nTA T0 and TA-nTA T1 (p=0.01802620), TA-nTA T0 and TA-nTA T2 (p=0.01409357) and TA-nTA T0 and TA-nTA T3 (p=0.03556224).

## Conclusions

These findings suggest a “physiological change associated to the use of Pho-S” that may be correlated to decrease in tumoral blood flow with consequential decrease in temperature associated to ischemia or necrosis.

# Immunohistochemical evaluation of caspase-3 and Ki67 expression in canine soft tissue sarcomas subjected to synthetic phosphoethanolamine (Pho-S) neoadjuvant therapy

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## Introduction

Synthetic phosphothanolamine (Pho-S) induces increased activity of caspase-3 and triggers cell death by apoptosis. The expression of Ki67 protein is strongly associated with cell proliferation. This study determines degree of apoptosis (caspase-3) and cell proliferation índex (Ki67) by immunohistochemical (IHC) analysis of histological cuts of untreated (G1) and Pho-S treated (G2) canine soft tissue sarcomas (STS).

## Materials and methods

Values obtained from tumors from the group that received four weekly chemotherapy sessions with Pho-S (70 mg/kg/IV) as neoadjuvant therapy before oncologic surgery (G2) were compared to values from the group that did not receive Pho-S neoadjuvant treatment (G1). Wilcoxon test was used for comparisons regarding caspase-3 and Student T test for comparisons regarding Ki67. Values lower than p

## Results

Immunohistochemistry comparison between untreated (G1=10) and Pho-S treated tumors (G2=9) showed no significant difference regarding degree of apoptosis (caspase-3) according to tumor grade I (p=0.363) and II (p=0.4543), and cell proliferation index (Ki67) according to tumor grade I (p = 0.6588) and II (p = 0.179). It was not possible to perform such comparison between groups for grade III STS because none of the sarcomas in G2 were classified as grade III. If we disregard the sarcoma grade (I, II, III) there was no difference in caspase-3 (p=0.7002) and Ki67 (p=0.7619) between G1 and G2.

## Conclusions

STS of dogs treated with Pho-S as described in this study showed no significant difference in expression of caspase-3 and Ki67.

# Immunotherapy with empty cowpea mosaic virus nanoparticles against canine oral tumors

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## Introduction

Oral tumors (squamous cell carcinoma, malignant melanoma and fibrosarcoma) represent ~7% of all canine cancers. Because these tumors have a high metastatic potential and there are not efficacious therapies, the patient outcome is poor. This study evaluated the safety of in situ administration of empty cowpea mosaic virus nanoparticles (eCMPV) in 3 dogs with oral tumors. The eCMPV are non-infectious, non-replicative, and highly immunogenic nanoparticles.

## Materials and methods

Three canine patients with oral tumors (two oral melanoma and one oral fibrosarcoma), received standard oral chemotherapy in combination with eCMPV (0.1–0.4mg) administered intratumorally weekly for a maximum of 6 weeks. Tumor biopsies and blood samples were collected before, during and after treatments. Weekly tumor measurements and computed tomography (days 1 and 35) were taken to evaluate tumor response using the RECIST.

## Results

No alterations in chemokines or cytokines in peripheral blood samples were observed in the eCPMV-treated patients. An increase in blood lymphocytes was observed in the dog with oral fibrosarcoma. After treatment, stable disease was observed in oral

fibrosarcoma patient (21 days) and oral malignant melanoma patient (28 days) followed by disease progression.

## Conclusions

Due to the biologic behavior and the invasive capacity of malignant oral tumors, the conventional treatments (surgery, chemotherapy and/or radiotherapy) usually lead to disease relapses. Our results demonstrated that the use of eCPMV as immunotherapy is a safe option to treat canine oral tumors, allowing more extensive trials with higher doses/frequency to improve outcome in canine patients.

# Safety and efficacy of oncolytic virotherapy in canine patients with gliomas

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## Introduction

The use of oncolytic virus (OV) is a growing field of interest since they are particularly helpful for the treatment of non-surgical tumors, such as high-grade gliomas. Incomplete surgical resection, radiation and/or chemotherapy achieve a poor short-term prognosis in gliomas, fortunately the use of OV shows promising results. Our goal is to determine the safety and efficacy of intratumoral administration of OV ICOCAV15 in canine intracranial gliomas.

## Materials and methods

The ICOCAV15 was administrated intratumorally in 6 dogs that presented gliomas through craniectomy. Blood samples were obtained previously and after treatment (from 1 to 365 days). To determine RAVNO criteria, follow-up by magnetic resonance imaging (MRI) was analyzed after 15, 60, 180 and 365 days.

## Results

In the first 24-48 hours after treatment high arterial pressure was observed in 4/6 patients. Regarding blood cells, a progressive increase of leucocytes until 9 days followed by a decrease to basal levels was observed. Three patients died due to progression disease (PD) at days 76, 176 and 318; two of them previously shown stabilization disease (SD) for 2 months and a complete remission (CR) for 6 months. Currently, one patient presented PD at 110 days, one patient achieved SD after 439 days, and the other one showed CR after 264 days of follow-up.

## Conclusions

Our results show that intratumoral administration of ICOCAV15 in canine gliomas is safe. Further, the increasing in the survival time due to the clinical response of some patients suggests that the role of ICOCAV15 should be further explored as a treatment in veterinary neuro-oncology.

# Comparison between radical and palliative radiotherapy in canine nasal tumours

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## Introduction

Radical-intention radiotherapy is considered the treatment of choice for canine sinonasal tumors. However, logistic or economic factors can often limit its application. The objective of this retrospective study was to compare median of survival time (MST) between radical and palliative radiotherapy protocol.

## Materials and methods

Data were collected from dogs with nasal carcinomas and sarcomas (staged following the modified Adams classification) treated with a 6 MV linac. The treatment was planned with a 3DCRT or IMRT approach. The prescribed dose for radical-intent protocol was 50-55 Gy (5Gy fractions, 4 days per week) and 27-32 Gy (8-9 Gy fractions, 1-2 days per week) for the palliative intent ones.

## Results

Thirty-one dogs were included in this study. Twenty-two carcinomas (22/31; 70.96%), 6 sarcomas (6/31; 19.35%) and 3 undifferentiated tumors (3/31; 9.69%). One dog had clinical stage I (1/31, 3.22%), 3 stage II (3/31; 9.67%), 12 stage III (12/31; 39.70%) and 15 stage IV (15/31; 47.41%). Twenty-six dogs (26/31; 81.3%) were treated with radical-intent and five (5/31; 15.6%) with palliative intent. MST for all treated dogs, regardless of the tumor type or clinical stage, was 582 days (+/-3.24). MST was 458 days (+/-91.3) for the radical intention treatment, and 499 days (+/-69.07) for the palliative intention one (p= 0.49).

## Conclusions

MST for the radical treatment was comparable to other published studies. However, MST for the palliative intention approach exceeded the results previously reported. Nevertheless, these results should be corroborated with a study involving a larger number of animals.

# Increased efficacy of combined treatment with chlorambucil and firocoxib on human and canine mammary tumor cell lines

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## Introduction

COX-2 overexpression has a key role in human and canine mammary carcinogenesis. In recent years, continuous lower dose (metronomic) chemotherapy together with non-steroidal anti-inflammatory drugs has been used against various tumors, including canine mammary carcinoma. Our group examined the effectiveness of combined firocoxib and chlorambucil treatment using human and canine mammary tumor cell lines.

## Materials and methods

IC50 concentrations of firocoxib and chlorambucil were determined in MCF-7 (human epithelial), CMT-U27 (canine epithelial) and CMT-U309 (canine mesenchymal) cell lines. The combined effect of the 2 drugs were investigated using a Hamilton Starlet liquid handling system. To determine the long-term effect of the compounds, cells were treated for 5 days in 12 well plates and the confluence (cell spreading) was compared till day 20. Moreover, DNA double strand breaks (DSBs) were quantified as determining the number of H2AX pixels colocalizing with DAPI by ZEN confocal software.

## Results

Firocoxib was not toxic up to 500uM, while after chlorambucil treatment confluence decreased to 70% in MCF-7 and CMT-U cells with 94uM and 25uM concentrations, respectively. After combined firocoxib and chlorambucil confluence was only 45%. Synergistic effect was observed when firocoxib was added at 300uM or higher concentrations. The number of H2AX pixels with DAPI colocalization in untreated cells (4 416) increased to 15 207 and 162 776 as a result of firocoxib and chlorambucil treatment, respectively, while in the case of combined treatment it reached 196 897.

## Conclusions

Our in vitro data suggest that combination therapy using chlorambucil and firocoxib may be more effective than the standard protocol.

# Caval chemodectoma in a cat: a case report

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## Introduction

An eleven-year-old male neutered Domestic Shorthair cat was presented with a 4-week-history of an intermittent cough and dyspnoea. A pleural effusion was identified which was confirmed as chyle.

## Materials and methods

Echocardiography and computed tomography revealed a 16mm mass cranial to the heart, which was invading the cranial vena cava . Because of the location of the mass, it was assumed that chylothorax had developed as a result of direct disruption of the thoracic duct by the tumour or secondary to central venous hypertension. An exploratory thoracotomy was performed and the mass, which originated within the wall of the cranial vena cava, was excised with narrow gross margins. Histopathology and immunohistochemistry were consistent with a chemodectoma with microscopic residual tumour cells. Given the residual microscopic disease, adjuvant treatment with toceranib phosphate was initiated.

## Results

The cat remained well for the duration of treatment and was euthanised 31 months after diagnosis when a computed tomography identified recurrent pleural effusion, a heart base mass with cranial vena cava and azygos vein invasion.

## Conclusions

Chemodectomas are rare in cats, with only 13 cases reported in the literature to date and all were located in either the aortic or carotid body. The reported survival with partial resection and/or subtotal pericardiectomy was 13-19 months. Treatment of feline chemodectomas with toceranib phosphate has not been previously reported. To the authors' knowledge, this is the first description of a surgical management of a feline vena cava chemodectoma, combined with adjuvant toceranib phosphate, resulting in a prolonged survival.

# Monitoring of the clinical and immune response to the combines treatment with electrochemotherapy and gene electrotransfer of plasmid encoding canine interleukin-12 in dogs with oral and mast cell tumors

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## Introduction

Electrochemotherapy (ECT) and gene electrotransfer of interleukin-12 (IL-12 GET) are used to treat different tumours in dogs. In order to improve and predict the therapeutic outcome as well as select the suitable patients, we evaluated the systemic immune response and the expression of the programmed death receptor and its ligand (PD-1 and PD-L1) in the tumours to define the immune response to ECT and IL-12 GET and possible predictive factors for the treatment response.

## Materials and methods

Fourteen dogs with mast cell tumours (MCT) and nine dogs with oral malignant melanoma (OMM), treated with ECT and IL-12 GET, were included in monitoring of the systemic immune response. Flow cytometry was performed for detection of CD4+, CD8+, and regulatory T cells (Treg) in blood before and after the treatment. In addition, before treatment, in tumours of 30 dogs (21 MCT and 9 OMM) the expression of PD-1 and PD-L1 was determined immunohistochemically.

## Results

The results show a decrease in the percentage of Treg in the course of treatment ($p<0.05$).

## Conclusions

In conclusion, a systemic immune response to the combined treatment was determined. The expression of PD-1 and PD-L1 could be used as a prognostic marker for dogs with MCT and OMM.

# Molecular evaluation of CSPG4 target in canine melanoma and osteosarcoma for novel therapeutic strategies

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## Introduction

Canine and human melanoma and Osteosarcoma (OSA) are aggressive tumors with common characteristics making dogs a good model for comparative oncology. Novel therapeutic strategies against these tumors could be useful to both species. In humans, chondroitin sulphate proteoglycan 4 (CSPG4) is a marker involved in tumor progression and could be a candidate target for immunotherapy. In order to assess this topic, we investigate the CSPG4 expression by RT qPCR, in canine melanoma and OSA.

## Materials and methods

Total RNA, extracted from tissue samples (melanoma n=16; OSA n=13; paired controls = normal tissues) was retro-transcribed and subject to duplex RT-qPCR using two different TaqMan assays for the target gene CSPG4 and the internal reference gene (RG) Ribosomal Protein S19 (RPS19). RPS19 was selected from a panel of 9 candidate RGs, according to NormFinder analysis. Relative expression was analyzed by CFX Maestro™ Software. Student t-test and ANOVA were performed (significance set at $P<0.05$)

## Results

We show that gene expression of CSPG4 in OSA tissues is significantly increased by 3-4 folds when compared to controls biological group, while in melanoma, although an increasing trend was observed, no significant differences between the two groups were highlighted. This result could be caused by the non-homogeneous expression of CSPG4 in cells within melanoma compared to OSA as the immunohistochemistry analysis of the two cancer types showed.

## Conclusions

The validation of CSPG4 immunotherapy marker in canine melanoma and OSA may have an impact to translate this strategy modality to human oncology.

# Evaluation of canine prostate and bladder cancer cell lines as models for COX 2 inhibition

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## Introduction

Prostate adenocarcinoma (PAC) in dogs is a highly malignant cancer without satisfying therapeutic options, whereas cyclooxygenase 2 (COX-2) inhibitors like meloxicam prolong survival in canine bladder transitional cell carcinoma (TCC). Cell lines provide valuable tools for in vitro evaluation of therapeutic approaches. However, clonal selection as well as cultivation itself can significantly change the cellular profile. Herein we comparatively characterized cellular origin and epithelial character of canine PAC and TCC cell lines and their respective original tumor tissue. Further, we evaluated the inhibitory effect of COX-2 inhibitor meloxicam on the cell lines.

## Materials and methods

Cell lines derived from six PAC, three TCC and two PAC metastases, as well as seven respective original tumor tissues were immunohistochemically stained with antibodies detecting pan CK, CK7, CK8/18, UPIII, E-Cadherin, vimentin and COX 2. After exposing the cells to 1 and 10 µM meloxicam, PGE2-secretion, metabolic activities and cell counts were assessed.

## Results

Despite changes in vimentin and pan-CK immunoreactivities, the overall staining pattern remained stable in cell lines compared to original tissues. COX 2 expression was confirmed in all samples. Meloxicam reduced PGE2-secretion in seven out of nine cell lines significantly at 1 µM. This was improved by 10 µM in only one cell line. Metabolic activity and cell counts remained unaffected.

## Conclusions

These nine cell lines are close to their respective original tumor tissue and proved their qualities as COX-2 in vitro models for canine prostate and bladder cancer. The inhibitory effect of meloxicam on COX-2 is saturated at 1 µM comparable to therapeutic plasma concentrations observed in dogs.

# Neoadjuvant 'In situ vaccination' immunotherapy induced tumor reduction and significantly improved survival in canine inflammatory mammary cancer. A case report

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## Introduction

Canine inflammatory mammary cancer (IMC) is the most aggressive type of mammary cancer, with minimal therapeutic options and surgery is contraindicated. In this study, in-situ vaccination using Cowpea mosaic virus-like particles (VLPs) which are empty non-infectious and non-replicative, highly immunogenic nanoparticles, was used in a dog with IMC.

## Materials and methods

An 11-year-old neutered female dog with bilateral IMC (2 nodules: T1 and T2) received neoadjuvant intratumor injection of VLPs (0.4 mg weekly for a total of 8 injections) plus anti-tumor therapy (anti-COX2, toceranib phosphate and low-dose-cyclophosphamide). Tumor size was measured to evaluate the tumor growth inhibition (TGI) rate induced by VLPs. Blood analyses, histopathology and immunohistochemistry were performed throughout the treatment. Quality of life (QoL) was assessed.

## Results

Therapy resulted in significant TGI (80% size reduction for T1 and 71% for T2) and surgery was indicated 106 days after diagnosis. In a previous series of cases, the mean survival time for IMC cases treated with chemotherapy was 57 days. A blood and tumor tissue neutrophilic response was observed. Pathological study revealed a c-Kit-, grade III lipid-rich carcinoma with triple-negative non-basal epithelial phenotype (panCK+, CK14-, vimentin-, calponin-, p63-, ER-, PR-, AR-, HER2-) showing intratumor T and B lymphocytic stimulation (CD3+, CD20+, respectively). Patient died due to kidney disease 187 days after diagnosis, with improved QoL and relapsed IMC.

## Conclusions

VLP immunotherapy resulted in a significant tumor reduction, improved survival and good QoL. This promising immunotherapy is currently being evaluated in additional inflammatory and non-inflammatory canine mammary tumors. to expand these remarkable findings.

# Vet-OncoNet – Portuguese Companion Animal Cancer Registry

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## Introduction

Veterinary Oncology Network – Vet-OncoNet is a pioneer institutional initiative that collects, uniformizes, and centralizes data of neoplasia in companion animals in Portugal.

## Materials and methods

The Net is based on a partnership of data and information exchange between Vet-OncoNet and several types of partners, among which veterinary diagnostic laboratories. The data are incorporated into a central database after extraction and cleaning. Each registry is classified according to the Global Initiative on Veterinary Cancer Surveillance (GIVCS) under construction system which is harmonized with ICD-O-3.2. Once treated, data are made available under the form of customized dashboards for each partner.

## Results

Since December 2019 we count 5856 entries, 80,4% canine, 18,9% feline. For dogs, peak age was 8-11 years old (YO), for cats ranges from 8-15 YO. Main dog topographies: skin (49%), mammary gland (MM) (21,4%), subcutaneous and soft tissue (SST) (8,8%), testis (4,2%); for cats: skin (40%), MM (34,7%), gastrointestinal tract (5,6%), SST (3,3%). For dogs, the most frequent tumour types were cutaneous mast cell tumor (10,1%), lipoma (5,3%) and complex adenoma of MM (4,3%). For cats, squamous cell carcinoma (12,2%), MM simple adenocarcinoma (8,1%) and lymphoma (6,7%) were the most common.

## Conclusions

This is the first animal cancer data report in Portugal and skin tumors followed by mammary neoplasms are the most prevalent cancers. Using a similar structure to the Portuguese Human Cancer Registry, this work will allow future human comparative studies and it is expected to be produced in the following years demographic and geographic-based information on animal cancer incidence in Portugal.

# Assessment of distant metastatic disease in Kiupel low grade / Patnaik intermediate grade canine cutaneous mast cell tumours

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## Introduction

Histologic grade is considered the most consistent and reliable method to predict the biological behaviour of canine mast cell tumours (MCTs). Previous reports suggest Kiupel low grade / Patnaik intermediate grade MCTs have a low metastatic rate to local lymph nodes and distant sites. The objective of this study was to determine the metastatic rate to distant sites of cutaneous MCTs (Kiupel low grade, Patnaik intermediate grade) at the time of initial evaluation and therefore the utility of staging. A secondary objective was to determine if any patient or tumour variables were associated with the presence of metastasis.

## Materials and methods

Medical records of dogs with cutaneous MCTs (low grade Kiupel, intermediate grade Patnaik) presenting for initial evaluation between 2012 and 2019 were retrospectively reviewed. Dogs were eligible for recruitment if they had staging with an abdominal ultrasound and cytological evaluation of the spleen and liver.

## Results

Forty-one dogs met the inclusion criteria of the study. The most frequently represented breeds were Labrador Retrievers (26.8%) and Golden Retrievers (14.6%). The most common primary tumour location was the trunk and perineal area (53.7%). Seventeen patients (41.5%) had the local lymph node assessed, with four (23.5%) having evidence of lymph node metastasis. No dog had evidence of spleen and/or liver metastasis.

## Conclusions

This study confirms, in accordance with previous reports, that cutaneous MCTs (Kiupel low grade, Patnaik intermediate grade) have a low metastatic rate to local lymph nodes and distant sites. The utility of staging with abdominal ultrasound including liver and spleen aspirates was low.

# Canine bladder urothelial carcinoma treated by intraoperative radiotherapy and COX-2 selective NSAID: a retrospective study of 16 cases (2014-2019)

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## Introduction

Urothelial carcinoma (UC) is the first most common urinary bladder cancer in dogs. Several therapeutic approaches are described and, surgery and chemotherapy are currently considered as the most reliable approach to improve the survival rate even if the prognosis remains poor. Intraoperative radiotherapy is an appealing approach to control the local aggressivity of such tumors, since 1987, however, only two studies have been published on the treatment by intraoperative radiotherapy of canine UC, making additional and recent data invaluable on that subject.

## Materials and methods

Medical records of dogs with bladder UC, histologically diagnosed, and treated by one-shot intraoperative radiotherapy at MICEN VET Oncology center and MICHEL BARON Surgery center and COX-2 selective NSAID were retrospectively reviewed. Reported data included CBC, serum biochemical analyses, urinalysis, thoracic and abdominal imaging including CT scan for measurement of UC, and complications.

## Results

Sixteen dogs were included in the study, the median survival time and median disease free time were 542 and 273 days respectively; the 1-, 2- and 3-years survival rates were 81%, 37%, 0% respectively. Several exposures, such as age, histological differentiation, and ureteral reimplantation had a significant negative impact on the survival time. Complications associated with the therapy occurred in 50% of dogs, and were considered as mild and self-limiting in half of these cases; long-term urinary incontinence occurred in 19% of the cases.

## Conclusions

The results of this retrospective cohort study suggest that one-shot intraoperative radiotherapy and COX-2 selective NSAID are relatively well tolerated and may be a treatment option for dogs with UC.

# Characterization of radio-sensitivity of canine oral malignant melanoma cell lines and their secretion of cytokines

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## Introduction

Irradiation has an important role in the therapy of canine oral malignant melanoma (COMM). We assessed the radio-sensitivity of primary COMM cell lines and the influence of irradiation on the secretome of these cells.

## Materials and methods

COMM cells explanted from primary lesions and a lymph node metastasis were irradiated with 0, 2, 4, 6, 8, and 10 Gy. Over a course of 7 days, cell proliferation was assessed by proliferation assay on a daily basis. Cell supernatants collected on days 0, 4 and 6 post irradiation with 0 vs. 6Gy were analyzed for cytokine expression via Luminex assay.

## Results

From 0 - 6 Gy, radio-sensitivity of COMM cells was dose-dependent. Higher irradiation doses did not further enhance this effect. Cells explanted from primary COMM responded well to irradiation, as shown by hypo-proliferation, whilst metastasis were rather resistant to radiotherapy. Transient irradiation-mediated deregulation was observed for IL-10, IL-8, and MCP1. Interestingly, irradiation induced significant down-regulation of VEGF secretion over the entire observation period.

## Conclusions

Overall, presented data provide evidence that primary COMM-derived cells are amenable to radiotherapy, whilst metastasis tend to resist irradiation. We also show that radiotherapy can modulate the tumour cell secretome. Whilst this effect was only transient for IL-10, IL-8, and MCP1, it proved long lasting for VEGF. These findings are suggestive for irradiation having an inhibitory impact on COMM vascularisation, and hence, may help preventing or retarding metastasis.

# Long term outcome with multimodality treatment in a cat with laryngeal adenocarcinoma

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## Introduction

Feline laryngeal adenocarcinoma (ACA) is rare and there is sparse information detailing treatment protocols and outcomes. Current case reports depict a guarded prognosis. However, definitive-intent, multimodal approaches have not been described. The purpose of this report is to describe the clinical presentation, treatment, and excellent outcome in a cat with histopathologically confirmed, recurrent, localized laryngeal ACA.

## Materials and methods

A 9.5 year-old male neutered domestic short-haired cat received two courses of postoperative, definitive-intent conformal radiation therapy (RT). Radiation was initially prescribed (16 x 3Gy, total 48Gy) following three surgical resections; the second protocol (20 x 2.5Gy, total 50Gy) was prescribed following tumour recurrence and two additional resections.

## Results

The mass recurred twice within 10 months following surgery alone. Following surgery and the first RT course, tumour control was achieved for 2.5 years. At the time of local recurrence, two additional cytoreductive surgeries were performed. The cat was retreated with RT and remains disease-free 22 months after the second course. Acute RT toxicity was mild with both courses and no clinically relevant late RT toxicities have developed to date.

## Conclusions

Feline laryngeal ACA is uncommon and optimal treatment paradigms are unknown. This is the first report of a cat with laryngeal ACA achieving long-term control following local surgery and definitive-intent RT. Overall, the cat has experienced greater than 5 years of excellent quality of life. Surgery and definitive-intent RT should be considered in feline patients with laryngeal ACA.

# Overall and relapse-free survival of dogs with appendicular osteosarcoma after limb-sparing surgery using de-immunized allografts coated with stromal mesenchymal cells: 25 cases (2013-2017)

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## Introduction

Appendicular osteosarcoma is the most common bone tumor in dogs. Without receiving specific combined treatment, the majority of patients are euthanized within 16-20 weeks after initial diagnosis due to metastatic spread or intractable tumor-induced pain. Despite certain beneficial effects of amputation, the radical surgery might be contraindicated in giant dogs, patients with orthopedic and neurological disorders or with excessive weight. In these patients, in particular, limb-sparing surgical techniques allow to save functional activity of osteosarcoma-affected extremities and sufficiently improve patients' quality of life.

## Materials and methods

We analyzed overall survival, surgical results and recurrence rate in dogs with histologically diagnosed appendicular osteosarcoma after limb-salvage surgery using de-immunized allogenic bone grafts coated with recipient's stromal mesenchymal cells. 25 dogs underwent a combined treatment with chemotherapy (cisplatin, 60-70 mg/m2, IV, 3-4 cycles) and wide segmental resection of the affected bone with an allograft defect replacement.

## Results

Overall survival in the group amounted to 321 days, and median progression-free - 222 days. Local relapse was diagnosed in 4 cases (16,6%), of which 50% were recorded within 150 days and the rest – after the two-year period. The overall functional outcome in the group was good.

## Conclusions

The combined limb-sparing protocol is an efficient treatment option for canine appendicular osteosarcoma, wherein limb-salvage surgery might be preferable in certain groups of patients due to its beneficial potential to improve mobility and quality of life.

# A clinical case of hypertrophic osteopathy in a cat with papillary renal cell carcinoma

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## Introduction

Hypertrophic osteopathy (HO) is a syndrome characterized by progressive periosteal proliferation that mainly affects long bones of thoracic and pelvic limbs. In most cases, HO is secondary to intrathoracic lesions of neoplastic, inflammatory or degenerative origin. Paraneoplastic HO is commonly associated with pulmonary tumors and rarely diagnosed in companion animals with abdominal malignancies. The pathoetiology of HO remains elusive with proposed mechanisms of vascular factors release, neurohumoral influence, increased peripheral blood flow, and proliferation of connective tissue.

## Materials and methods

A 14-year-old neutered male cat was admitted to the clinic with bilateral lameness and soft tissue swelling on the distal parts of all extremities. Clinical examination and abdominal ultrasonography detected a tumor of left kidney (3x4 cm) while computer tomography confirmed diagnosis of the malignant growth with no presence of metastasis. Radiographs revealed periosteal bone formation on the long bones of all four limbs. The cat underwent radical left nephroureterectomy. At histopathological examination of the affected kidney papillary renal cell carcinoma was observed.

## Results

During two-month period after the surgery clinical improvement in mobility, pain relief were noted in the patient. At a follow-up of eight months neither metastatic dissemination nor local recurrence was detected.

## Conclusions

The present clinical case shows beneficial effect of surgical excision of primary tumor in alleviating clinical signs of paraneoplasic HO. Papillary renal cell carcinoma should be included in the list of underlying oncological diseases associated with HO in cats.

# Glucoregulatory factors in canine hepatocellular carcinoma and leiomyosarcoma with nonislet cell tumor hypoglycemia (NICTH)

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## Introduction

Hypoglycemia caused by malignant tumors except for insulinoma is called nonislet cell tumor hypoglycemia (NICTH). It is reported that hepatocellular carcinoma (HCC) and leiomyosarcoma (LMS) may cause NICTH: however, its pathogenesis is still unclear. Therefore, this study aimed to evaluate the gene expressions of glucoregulatory factors in canine HCC and LMS with hypoglycemia.

## Materials and methods

Eight dogs with HCC and 3 dogs with LMS were included in this study: 2 dogs (cases #1 and #2) with HCC and 1 dog (case #3) with LMS were hypoglycemic. All dogs underwent surgery. In dogs with hypoglycemia, blood glucose levels were compared between preoperative and postoperative periods. The resected tumors were used for the comparison of gene expressions of insulin-like growth factor (IGF)-1 and 2, IGF-binding protein-3 (IGFBP-3), insulin, glucagon and gastric inhibitory polypeptide (GIP).

## Results

Preoperative blood glucose (27, 63 and 32 mg/dL in cases #1, #2 and #3, respectively) increased after the operation. Compared with the dogs without hypoglycemia, the gene expression of IGF-1 was 4.28, 0.14 and 1.14-fold in cases #1, #2 and #3, respectively, whereas that of IGF-2 markedly increased 10.8, 31.77 and 760.1-fold in cases #1, #2 and #3, respectively. In addition, the gene expression of IGFBP-3 was no difference between the dogs with and without hypoglycemia, and those of insulin, glucagon and GIP was not detected in any of the dogs.

## Conclusions

The overexpression of IGF-2 is suggested to cause NICTH in dogs with HCC and LMS.

# Not always benign! – Histopathological diagnosis of neoplasms in pediatric dogs

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## Introduction

Tumors are predominantly present in old animals. The aim of this study was to describe which types of tumors occur in juvenile dogs up to 12 months of age.

## Materials and methods

Data sets from histopathological routine investigations submitted to LABOKLIN GmbH & Co. KG (2016–2019) were analyzed retrospectively. Included were samples from 7243 dogs where breed, age (up to 12 months), and final diagnosis were reported.

## Results

The most common breeds were mongrel (n=1482), French bulldog (n=654), and Labrador retriever (n=464), regardless diagnoses. Non-neoplastic lesions were found in 59%, and tumor-like lesions in 3% dogs. Furthermore, 2750 tumors (38%) were identified. Neoplasms from 2451 dogs were benign (89%). Histiocytoma was most frequent (n=1896, 77%) with French bulldogs overrepresented (n=248). Other benign tumors were papilloma (n=283), hair follicle tumor (n=79), and mammary tumor (n=53). (Semi)Malignant tumors had 299 dogs (11%) including mast cell tumor (n=87), lymphoma (n=24), melanoma (n=7), soft-tissue sarcoma (n=33), bone tumor (n=13), other sarcoma (n=30), mammary tumor (n=26), other carcinoma (n=35), anaplastic neoplasia (n=21), and miscellaneous (n=33). From mast cell tumors, 3% were grade 3/high-grade, and 6% of soft-tissue sarcomas were grade 3. Lymphomas were located in lymph nodes (42%), gastrointestinal tract (29%), spleen/liver (21%), or skin (8%). In 82 submitted bone samples, 14 cases with bone-derived neoplasms were identified which were mostly malignant (93%).

## Conclusions

The pediatric dogs showed predominantly benign tumors, especially histiocytomas. However, 11% of the tumors were (semi)malignant. Therefore, cytological or histopathological examination of lumps in juvenile dogs are recommended because of the therapeutic and prognostic relevance.

# Evaluation of novel bioabsorbable calcium sulfate beads for local delivery of platinum: In vitro elution study

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## Introduction

Carboplatin drug-delivery systems allow high sustained release of platinum with limited systemic toxicity for local tumor control after incomplete or marginal excision. The current commercialized drug-delivery system is non-FDA approved and associated with high compounding variability. A novel kit of resorbable calcium sulfate beads is marketed specifically for use in veterinary medicine and local delivery of antimicrobials. The purpose of this study was to evaluate its use as a carrier for carboplatin, characterize the carboplatin elution in vitro, and investigate whether the initial dose and formulation of carboplatin, or the bead size significantly influences the rate of carboplatin elution.

## Materials and methods

An in vitro elution study was performed with five doses and two formulations of carboplatin (20, 50, 100, and 500 mg carboplatin per kit in powder and liquid formulations). Beads were placed in 37°C PBS solution for 72 hours, and carboplatinum concentrations in the eluent were measured by high-performance liquid chromatography (HPLC) at 11 time points with a modified United States Pharmacopeia (USP) assay.

## Results

Concentrations of carboplatin in the eluent proportionally increased with the initial dose and peaked at 48 hours, ranging from 48.7 to 72.3% of the initial load. Higher peak concentrations ($p = 5x10-4$) and elution rates ($p = 3x10-9$) were observed with the liquid formulation. There was no significant difference in maximal concentration and peaking time between 3- and 5-mm diameter beads ($p = 0.797$, $p = 0.325$).

## Conclusions

The novel kit can be used for preparation of carboplatin-impregnated resorbable calcium sulfate beads at variable doses, sizes and formulations.

# Balloon cementoplasty with neoadjuvant radiation therapy for palliation of appendicular osteosarcoma in 5 dogs: perioperative and short-term clinical outcomes

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## Introduction

Percutaneous cementoplasty provides pain relief, restores mechanical strength, and prevents pathologic fractures in humans suffering osteolytic bone lesions. Promising functional outcomes have been reported in a canine case series, despite perilesional cement leakage and pulmonary thromboembolism. This prospective study aimed to evaluate the technical feasibility and safety of balloon cementoplasty and palliative-intent radiation therapy as an innovative interventional limb-sparing approach to canine appendicular osteosarcoma.

## Materials and methods

Five dogs diagnosed with appendicular osteosarcoma (2 proximal humeral and 3 distal radial lesions) underwent balloon cementoplasty immediately after the second of two planned consecutive daily radiation fractions. Complications were recorded. Imaging, clinical and functional outcomes were evaluated over one month by computed tomography, subjective pain scoring and owner questionnaires, and objective gait analysis.

## Results

Technical feasibility and amount of cement injected varied between patients; feasibility appeared independent of anatomic location and degree of osteolysis. Perilesional cement leakage occurred in all patients without known complication. No fractures, thromboembolism, infection, or acute radiation side effects were observed. Body weight distribution to the affected limb was improved at 1 month post-treatment ($p = 0.012$). Peak vertical force ($p = 0.630$) and vertical impulse ($p = 0.9154$) symmetry indexes at 1 month were no different (no improvement; no deterioration) from baseline. Owners subjectively reported improved quality of life. All patients were alive at 1 month.

## Conclusions

Balloon cementoplasty appears safe and technically adaptable in combination with radiotherapy for canine appendicular osteosarcoma. Further testing and optimization

will aid in: (1) identifying patient selection criteria; and (2) improving clinical efficacy of the procedure.

# A multimodality radioimmunotherapy approach to treating advanced stage cancer in companion dogs

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## Introduction

Metastatic cancer carries the implication of incurability. Yet in the age of immunotherapy, a minority of patients develop immune responses that result in durable tumor regression. In efforts to expand this minority, we investigate external beam radiation (EBRT) and an intra-tumoral immunocytokine to create an in situ vaccine in one tumor microenvironment and combine this approach with systemic targeted radionuclide therapy (TRT) in order to propagate an abscopal effect at metastatic sites. We have previously documented durable responses (and cures) in rodent models and now present bridging data in companion dogs with advanced cancer.

## Materials and methods

Six dogs with melanoma or osteosarcoma received an 8 Gy dose of EBRT to their primary tumor and 3 daily intratumoral injections of hu14.18 immunocytokine. Concurrently, a tumor-targeting alkylphosphocholine (NM600) that chelates radionuclides was used as a ‘theranostic’ for serial diagnostic 86Y-NM600 PET/CT imaging of metastasis. These images enabled Monte Carlo dosimetry calculations for subsequent delivery of therapeutic 90Y-NM600. Adverse event (AE) data and biospecimens were collected throughout follow-up.

## Results

All metastatic tumors had differential uptake of 86Y-NM600 as evidenced by PET/CT. A minimum immunomodulatory dose of 2 Gy 90Y-NM600 was successfully delivered to all metastatic sites. Treatments were well tolerated and AEs were transient/low grade.

## Conclusions

We confirm tumor-selective uptake and the theranostic potential of NM600 to stage, create subject-specific dosimetry, and safely deliver TRT to metastatic sites in companion dogs with osteosarcoma and melanoma. Ongoing investigations include characterizing immunomodulatory and anti-tumor responses in larger cohorts along with investigations of higher TRT dosing and alternative radionuclides.

# Testing multidrug resistance (MDR) function in canine B-Cell lymphoma, mast cell tumor and mammary gland tumor

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## Introduction

One of the causes of MDR is drug efflux pump, i.e. P-glycoprotein (Pgp). Pgp function can be analyzed by flow cytometry (FACS). Different tumour types might show different characteristics of efflux function.

## Materials and methods

Neoplastic cells obtained from canine B-cell lymphoma (BCL) (n=23), mast cell tumour (MCT) (n=23), mammary gland tumour (MGT) (n=25). Samples were tested using Calcein-test by FACS analysis. Multidrug resistance activity factor (MAF) and the percentage of efflux active cells were calculated. Histopathology samples were graded according to Valli's, Patnaik's, Kiupel's and Goldschmid's grading system. Dogs with MCT, and MGT were treated by surgery, chemotherapy, or both. Lymphoma cases were treated by CHOP based protocol.

## Results

MAF values: BCL:0.19, ±0.17; MCT:0.17, ±0.13; MGT:0.13 ±0.17. BCL: age inversely correlated with MAF (r=0.42, p=0.04); MAF >0.2 was predictive for shorter overall median survival time (MAF0.2: 38 days, p= 0.0027) and disease free period (DFP). MCT: negative correlation was found with chemotherapy pre-treatment and MAF (r= -0.47); grade III cases had lower MAF than Grade I and II (p= 0,03). MGT: normal MGs showed less MAF, than tumors (0.03 ±0.05; 0.13 ±0.17, respectively; p=0.012), clinically worst MGTs showed higher MAF values than with better prognosis (35 ±0.18; 0.05 ±0.06, respectively; p= 0.027); higher grade (r=0.47) and stage (r=0.63) correlated with cells having increased pump function.

## Conclusions

MDR function analysis by FACS is fast and helpful to predict survival and relapse in dogs with BCL; seems to be useless with MCT (high grade types have low MAF values) and predicts MGT progression.

# Characterization of feline spontaneous malignant mammary tumors – a 10 year multicenter retrospective study in Portugal

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## Introduction

Most feline mammary tumors are malignant and show aggressive behavior. This study aimed to perform a retrospective characterization of feline spontaneous malignant mammary tumors (FMMT) presented over a 10 year period in Portugal.

## Materials and methods

116 queens with mammary masses were evaluated, most were of domestic shorthair (DSH) breed (n=109). 98 (84.5%) presented mammary tumors, of which 95 (96.9%) malignant and 3 benign neoplasms. 18 cats had non-neoplastic lesions.

## Results

FMMT were mostly DSH (n=90). Most were intact (64.1%) at diagnosis and had progestogens administration (78.6%). 37 cats (38.9%) had multiple nodules, in a total of 114 tumors. 17 animals had two (n=15) or three concomitant tumors of different histological subtypes. Abdominal glands were affected more frequently (56.8%). 34 cases had ulcerated masses at presentation, and 44 queens had at least one nodule >3.0 cm. Most queens were in clinical stage III (61.3%) and 11 in stage IV. Of the cases with follow-up, 76% developed local recurrence and 46% lung metastasis. Anemia, leukocytosis and leucopenia occurred in 26.7%, 20% and 8.9% cases, respectively. Tubulopapillary (48.2%), solid (33.3%) and cribriform (13.2%) carcinomas were the most frequent histological subtypes, and 51.9% were histological grade III. Most carcinomas showed infiltrative behavior, 37.7% had lymphovascular invasion and 43.2% of cases had lymph node invasion at diagnosis. Mean survival of queens with tumor-related death was 13.8 months (SD 12.7 months), while mean disease-free interval was of 9.1 months (SD 10.3 months).

## Conclusions

Tumor characterization is of utmost importance to identify risk factors of the disease and define preventive measures.

# Biomarkers of oxidative status in feline lymphoma

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## Introduction

The oxidative status of the organism is dependent of the balance between oxidant reactants and antioxidant defences. Oxidative stress develops when the oxygen and nitrogen free radicals exceed the capacity of the antioxidant defences of the organism. In humans and dogs, lymphoma was associated with the development of oxidative stress and its related damage. To our knowledge, information regarding the oxidative status in feline lymphoma is lacking. Therefore, the aim of this study was to investigate the antioxidant response in cats with lymphoma.

## Materials and methods

Serum concentrations of total serum thiols (Thiol) and total antioxidant capacity (TAC) were evaluated. TAC was determined by different methods, namely trolox equivalent antioxidant capacity (TEAC), ferric reducing ability of plasma (FRAP) and cupric reducing antioxidant capacity (CUPRAC). All antioxidants were determined in serum of 30 cats with lymphoma at diagnosis and prior to therapy, and compared with 12 healthy control cats. The diseased group was composed of cats with lymphomas in different anatomical locations and in different clinical stages.

## Results

Cats with lymphoma presented serum concentrations of Thiol (P

## Conclusions

The results obtained in the present study suggest that, as in humans and dogs, oxidative damage also occurs in cats with lymphoma, leading to a depletion in antioxidant defences. In addition, our results also suggest that analytes of oxidative status might be useful clinical biomarkers of this disease.

# Warburg effect in feline lymphoma

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## Introduction

The oxidative phosphorylation is the principal metabolic pathway of glucose in healthy cells. However, tumor cells use glycolysis - Warburg effect, as the preferred pathway of glucose metabolism, even under normoxemia. Although less efficient, aerobic glycolysis is a faster pathway to obtain energy, originates an increase in lactate and other metabolites that are important for carcinogenesis, and provides changes in the tumor microenvironment that favor tumor proliferation. The Warburg effect was described in different tumors in human medicine, including lymphoma. To our best knowledge, the Warburg effect was not evaluated in feline lymphoma.

## Materials and methods

The main objective was to evaluate the Warburg effect in cats with lymphoma. Thirty seven cats were enrolled in the study: 27 cats with lymphoma and 10 healthy control animals. Serum concentrations of glucose, fructosamine, lactate and lactate dehydrogenase (LDH) were determined in serum samples of all animals, collected at diagnosis and prior to therapy institution.

## Results

Diseased cats presented significantly higher serum lactate than controls (P

## Conclusions

These results suggest that the Warburg effect might be implicated in carcinogenesis of feline lymphoma, and also that lactate and LDH might be clinically useful biomarkers of the disease. Future studies are needed to evaluate the influence of different tumor features in concentrations of these biomarkers, and also to assess changes in glucose metabolism as therapeutic targets of feline lymphoma.

# Adenosine deaminase and uric acid in feline lymphoma

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## Introduction

Adenosine deaminase (ADA) has been used as a marker of cell-mediated immunity and chronic inflammation in human and in veterinary medicine. Its activity has been reported to increase in inflammatory, immune-mediated and neoplastic diseases. ADA is also implicated in the metabolism of purines, being responsible for catabolism of adenosine which will originate uric acid. The aim of this study was to investigate the serum activity of ADA and uric acid in cats with lymphoma.

## Materials and methods

Serum concentrations of ADA and uric acid were determined in serum of 30 cats with lymphoma at diagnosis and prior to therapy, and in 20 healthy control cats. The lymphoma group was composed mainly by domestic short-hair (DSH) cats, with ages ranging from 0.5 to 17 years and with lymphomas in different anatomical locations and in different clinical stages. Cats from the control group were also mainly DSH, with ages ranging from 2 to 13 years.

## Results

Cats with lymphoma presented serum concentrations of ADA (median 29.52 U/L, IQR 18.98-62.38 U/L) and uric acid (median 0.23 mg/dL, IQR 0.15-0.34 mg/dL) significantly higher (P

## Conclusions

The results obtained in our study revealed increased serum concentrations of ADA and uric acid in feline lymphoma cases, suggesting that these analytes might be useful clinical biomarkers of this disease.

# Evaluation of prognostic factors of feline spontaneous malignant mammary tumors

Hugo Vilhena [1, 2, 3], Joana Jesus [4, 5], Patrícia Dias-Pereira [6], Manuel Ramiro Pastorinho [7], Hélder Craveiro [1, 2], Ana Canadas-Sousa [6], Ana Catarina Figueira [1, 8]
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## Introduction

Mammary tumors are among the most common neoplasms in cats. This retrospective study aimed to evaluate potential prognostic factors of feline malignant mammary tumors (FMMT).

## Materials and methods

Queens presented with mammary masses over a 10 year period (n=116) were evaluated. Ninety-eight (84.5%) queens presented mammary tumors, of which 95 (96.9%) were malignant. Eighteen cats had non-neoplastic lesions. The influence of 21 potential prognostic factors – age, reproductive status, age at spay surgery, progestogen administration, number of tumors, location of affected mammary glands, tumor ulceration, tumor size, distant metastasis, clinical stage, type of surgery, surgical margins, location of tumor recurrence, type of tumor growth, tumor necrosis, histologic subtype, lymph node invasion, lymphovascular invasion, mitotic counts, nuclear pleomorphism and histologic grade – in FMMT disease free interval (DFI) and overall survival (OS) was evaluated. Queens submitted to adjuvant chemotherapy, with multiple tumors with different histological subtypes and which died of post-surgical complications were excluded.

## Results

Of the 95 queens with malignant tumors, 67 were eligible for follow-up analysis. Tumor size, clinical stage, surgical margins, type of tumor growth, lymphovascular invasion, nuclear pleomorphism and histologic grade significantly influenced DFI and OS. Additionally, lymph node neoplastic invasion, distant metastasis, type of surgery and tumor necrosis also significantly influenced OS. Multiple regression of combined contribution of the different parameters analyzed in OS presented a good correlation coefficient (R2=0.98 and adjusted R2=0.90).

## Conclusions

FMMT are associated with a poor prognosis. Prognostic factors identified in this study might be clinically useful to predict the evolution of the disease and to define therapeutic strategies.

# Spinal meningeal granular cell tumour in a dog treated with definitive radiotherapy

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## Introduction

Spinal granular cell tumours (SGCT) are rare in dogs. Although they have a characteristic histopathological appearance, their histogenesis remains uncertain. They can be locally infiltrative but have not been reported to metastasize. There is limited information regarding imaging features, treatment and prognosis in dogs with SGCT. Radiotherapy (RT) has been described as a therapeutic modality for SGCT in humans, but has not been evaluated in dogs.

## Materials and methods

A 10-year-old female neutered Samoyed was presented for investigations of progressive paraparesis, severe ataxia and faecal incontinence. MRI revealed a contrast enhancing intradural-extramedullary, broad-based lesion extending from the caudal T1 to caudal T3 vertebrae, asymmetrically occupying the full circumference of the spinal cord. Histopathologic findings of an incisional biopsy, including periodic acid-Schiff staining with diastase-resistance, were consistent with a SGCT. The dog was treated with CT-based 3D conformal RT (20 x 2.3 Gy to 46 Gy). Prednisolone was administered during RT, and tapered within 2 months. Toxicity and response scoring were evaluated by VRTOG and RECIST criteria, respectively.

## Results

Marked clinical improvement was noted post-RT. No acute toxicities were detected. At 1-year post diagnosis the dog showed mild residual ataxia and stable disease based on CT. No late toxicities have been documented to date.

## Conclusions

This is the first case reported of a dog with a SGCT circumventing the full diameter of the spinal cord, and the first described canine SGCT treated with RT. RT may provide clinical benefit to dogs with SGCT when surgical excision is not feasible.