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PROCEEDINGS

27th - 29th March 2009

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2009 Annual Congress
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Key Note Speakers

Friday 27th March 13:45 – 14:35
"Assessing efficacy of therapy by HRQL"
Ms Sabina M. Gasper, MA, MPH
Senior Director, Outcomes Research, Pfizer Animal Health, Inc

Sabina M. Gasper, MA, MPH is the Senior Director, Outcomes Research & Key Brand Clinical Support, US Operations, Pfizer Animal Health. She manages a team of Outcomes Research Scientists and clinicians who provide economic and clinical support to the Livestock and Companion Animal groups at PAH. Prior to joining Animal Health, Sabina spent 11 years on the human side working on health economic and outcomes research projects for various products, including anti-infectives and anti-fungals. She also worked on the development of the quality of life instrument used to measure patient QoL for Viagra. At PAH, Sabina is developing the Outcomes Research function to address the unique needs of the veterinary profession.

Saturday 28th March 10:00 – 11:00
"Surgical Oncology – Past, Present … but where is the Future"
Prof Dick White PhD, FRCVS DACVS DECVS
Principal, Dick White Referrals, UK

Professor Dick White is one Europe's leading small animal soft tissue surgeons. He spent almost 30 years teaching clinical surgery before founding his own specialist centre. He was on staff at the University of Cambridge for 25 years and was a Visiting Associate Professor at the University of Tennessee. Whilst in the US he became a Diplomate of the American College of Veterinary Surgeons and on his return, he joined forces with several other European surgeons to found the European College of Veterinary Surgeons and served as its second President.

His areas of special interest in soft tissue surgery include oncologic and reconstructive surgery. He has developed a number of novel oncologic surgical techniques and has published widely in all aspects of surgical oncology including editing the first BSAVA Manual of Clinical Oncology.

In 2003, he left academia and opened a specialist small animal centre in the UK. The centre has twelve consultant specialists, an active Intern and Residency programme and more than 80 staff.

Dick is an Honorary Life Member of the Veterinary Society of Surgical Oncology. He is also Professor of Small Animal Surgery at the new University of Nottingham Veterinary School where he contributes to the undergraduate teaching course.

Saturday 28th March 13:30 – 14:20
"*Targeted" Small Molecules in the Therapy of Cancer*
James M.G. Larkin, MD
Consultant Medical Oncologist, Dept of Medicine, Royal Marsden Hospital, Surrey, UK

Dr James Larkin gained his medical degree in 1996 from the University of Oxford. After completing general medical training he was awarded a PhD from the University of London in 2005 for his thesis 'Modelling Hyperacute Rejection as a Therapeutic Approach to Cancer'. He trained in medical oncology at the Royal Marsden and St Bartholomew's Hospitals and was appointed Consultant Medical Oncologist at the Royal Marsden Hospital in 2008. He specialises in the treatment of skin cancers including melanoma and in the treatment of renal cell carcinoma. His major research interest is in the use of biomarkers to tailor treatment for individual patients.

Sunday 29th March 09:45 – 10:45
"Radiopharmaceuticals in Diagnosis & Therapy of Cancer"
Dr Győző A Jánoki
Director, Medi-Radiopharma Ltd

Dr Gyozo A. Janoki studied at the Semmelweis Medical University, Budapest and gained his Doctor of Pharmacy in 1978. He became a Specialist in Radiopharmacy in 1994 and obtained his PhD in 1996. From 1975 to 1998 he was a lecturer and Head of Department of Applied Radioisotopes at the Frédéric Joliot-Curie National Research Institute for Radiobiology and Radiohygiene. In 1998 he took up the post of Deputy Director and Head of Department for Radiation Pathology and Medical Application of Radioisotopes at the Fodor József National Center of Public Health Frédéric Joliot-Curie. In 2005 he became Head of Department of Nuclear Medicine.

In 1997 he founded Medi-Radiopharma Ltd a GMP-certified radiopharmaceutical enterprice that produce over 30 in vivo diagnostics for the local and global (more than 40 countries) market. More recently he has found Radiopharmacy Ltd a GLP-certified contract laboratory that has a great potential to study radiolabelled ligands in vitro and in vivo.

Dr Janoki has published widely in the field of nuclear medicine. He became a member of the Hungarian Society of Nuclear Medicine in 1975 and held various executive posts including most recently President. He is a board member of the College of Nuclear Medicine of the Hungarian Academy of Sciences. He has received several awards including the George Hevesy Memorial Medal of the Hungarian Society of Nuclear Medicine.

He has many fruitful contacts with human medical doctors working in the field of Nuclear Medicine. He is also a great friend, clever motivator and generous supporter of veterinarians specialising in Veterinary Nuclear Medicine and Veterinary Oncology.

Resident Abstracts

Doxorubicin efflux is mediated through MRP-1 rather than P-gp in a canine leukaemia cell line.

M. Zandvliet[1], J.A. Schrickx[2], E. Teske[1] and J. Fink-Gremmels[2]

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Introduction: Tumour cell resistance to chemotherapeutic agents is the main reason for treatment failure in canine malignant lymphoma. Resistance to doxorubicin, one of most potent chemotherapeutic agents available, is associated with resistance to other chemotherapeutic agents known as Multi-Drug Resistance (MDR). One of the possible mechanisms of MDR is overexpression of drug-transporters of the ATP-Binding Cassette superfamily.

Materials and Methods: The GL-1 cell line, a canine B-cell leukaemia cell line, was transformed into a doxorubicin-resistant subline (GL-40) by continuous incubation with gradually increasing concentrations of doxorubicin. The expression of Pgp, MRP-1 and BCRP was measured by quantitative rt-PCR analysis and functionally with rhodamine123, calcein-AM and pheophorbide A, their typical inhibitors PSC833, MK571 and Ko143 and doxorubicin. Cellular retention of fluorescent substrates and doxorubicin was analysed by FACS after 30 minutes of incubation with or without inhibitors.

Results: The expression of Pgp increased (~7000x), MRP-1 decreased and BCRP remained unchanged in GL-40 compared to GL-1. Rh123, but not doxorubicin, retention decreased in GL-40. PSC833 increased Rh123 and, mildly, doxorubicin retention in GL-40. MK571 mildly increased doxorubicin retention in GL-1 and GL-40.

Conclusions: GL-1 and GL-40 express Pgp and MRP1. Pgp expression, but not MRP1, increased in GL-40. Doxorubicin retention was comparable between GL-1 and GL-40, suggesting that Pgp is not involved in doxorubicin efflux. MK571 mildly increased doxorubicin retention. These data indicate that doxorubicin is no substrate of canine Pgp, but rather of MRP1 and possibly that the mechanism of doxorubicin resistance in the GL-40 cells is independent of ABC-transporters.

Beyond histiocytoma: a retrospective study on tumours in young dogs

J.M. Schmidt[1], S.N. North[1], K.P. Freeman[2], F. Ramiro-Ibañez[2]

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The purpose of this study was to identify tumours occurring in young dogs based on biopsies submitted to Idexx Laboratories (UK).

The database was searched for diagnoses of tumours in dogs 12 months old or younger between 1993 and 2008. Tumours according to the World Health Organisation Classifications of Tumours of Domestic Animals were included. Excluded were uncertain diagnoses, "tumour-like lesions" and tumoural non-neoplastic conditions (e.g. calcinosis circumscripta).

20,280 histologic submissions from dogs up to 12 months were identified. 9537 submissions (47%) revealed a total of 9,546 neoplasias that fulfilled the search criteria. 8,465 (89%) were histiocytomas. In the remaining tumours (n=1,081; 11%) the largest group was benign epithelial tumours (n=375; 35%), which consisted mostly of cutaneous and mammary tumours. Haematopoietic tumours were the second largest group (n=229; 21%) including mast cell tumours (n=159), lymphomas/leukaemias (n=61), plasma cell tumours (n=5), histiocytic sarcomas (n=1) and unspecified haematopoietic tumours (n=3). The remaining tumours, in decreasing frequency, were benign mesenchymal tumours (n=145; 13%), non-hematopoietic malignant mesenchymal tumours (n=118; 11%), mixed, undifferentiated or unspecified tumours (n=80; 7%), malignant epithelial tumours (n=72; 7%), melanocytic tumours (n=42; 4%), or miscellaneous tumours (n=20; 2%).

Tumours other than histiocytoma were found in only approximately 5% of total submissions (11% of tumours) in dogs up to 12 months of age. Benign epithelial tumours and haematopoietic tumours represented more than 50% of them.

Immunohistochemical expression of VEGF in Canine Mammary Tumours

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Although several histopathological and clinical aspects of canine mammary gland tumours (MGT) have been widely studied there are considerable variations in its biologic behaviour hampering the definition of accurate prognostic factors. It has been suggested that it is the manifestation of several alterations in cellular physiology that collectively dictate malignant behaviour; sustained angiogenesis has been suggested as one of the most important of those characteristics. This process involves several growth factors, but many scientists believe that Vascular Endothelial Growth Factor (VEGF) is the most relevant one.

The aim of this investigation was to study, using immunohistochemical analysis, VEGF expression in canine mammary tumours (CMT) (64 malignant and 48 benign) and to understand if it is correlated with clinical (e.g. size and tissue fixation) or histopathological (e.g. type, growth, necrosis, lymphoid infiltration, lymph node metastasis, histological grade, E-caderin expression and proliferation index) factors with demonstrated prognostic value. The immunohistochemical assessment was based on the estimated percentage of neoplastic cells with cytoplasmatic labelling and on the staining intensity.

Statistical analysis did not show significant correlations between VEGF expression and clinico-phatological factors, suggesting that VEGF expression occurs in both malignant and benign tumours and is independent of histological type, proliferation, tissue invasion or local metastatic capacity.

These results suggest that VEGF expression is not related to the progression of MGT but survival studies are needed to confirm this hypothesis. The high prevalence of VEGF expression suggests, on the other hand, that there is a place for VEGF inhibitors in the treatment of CMT.

Making therapeutic decisions in canine appendicular osteosarcoma: Importance of CT scan and scintigraphy combination

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Lung and bone are primary sites of canine appendicular osteosarcoma (OSA) metastases. The purpose of this study is to describe resulting images of scintigraphy and CT scan to explore skeletal and pulmonary OSA extension; and to evaluate the complementarity between these two techniques.

Retrospective study. Included were dogs referred to evaluation of bone and pulmonary metastatic disease after OSA diagnosis. Planar scintigraphy images were obtained three hours after ^{99m}Tc-HMDP injection. Secondary areas of abnormal skeletal uptake and lungs were investigated with a CT scan.

Twenty-three dogs were included. Ten dogs had neither secondary areas of increased uptake nor suspect pulmonary images. Five dogs presented images compatible with pulmonary metastasis at CT scan. Ten dogs showed abnormal skeletal uptake in scintigraphy. Two of them had secondary areas of increased uptake compatible with degenerative joint disease at CT. Another dog exhibited a metacarpal uptake coincident with a fracture in CT scan. In five dogs, secondary areas of increased uptake were identified as lytic areas in CT, suggesting metastasis. In 4 of them, there were not suspect pulmonary images in CT scan. Lastly, two dogs presented abnormal uptakes without any identifiable lesion in CT scan.

In only 2 of 23 animals CT scan did not allow to characterize the suspect secondary uptakes visualized in scintigraphy. The well-known precocity of scintigraphy invites to retain and verify these areas with successive biopsies or CT scans. Scintigraphy and CT scan techniques should be combined to evaluate bone and pulmonary extension in cases of appendicular OSA.

FGFR2, MAP3K1, BRCA1 and BRCA2 are associated to Canine mammary tumor risk in dogs.

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Background: Breast cancer is a major contributor to overall morbidity and mortality in women. Several genes predisposing for breast cancer have been suggested, but the majority remain unknown. Less is known about the inherited risk factors underlying canine mammary tumours (CMT). CMT shows clear breed predispositions, with 36% of English Springer Spaniels (ESS) in Sweden being affected. The objective of this study was to evaluate ten genes, previously described in people for association with canine mammary tumours.

Methods: Ten genes (FGFR2, TNRC9, MAP3K1, LSP1, BRCA1, BRCA2, TP53, ERRB2, RCAS1, CHEK2) were selected. Sixty-three SNPs (4-9 SNPs per gene) were genotyped by iPLEX in 96 CMT cases (67 benign and 27 malignant, based on histopathology) and 96 controls from the ESS breed. The association analysis was performed with the program PLINK. $P< 0.01$ was considered statistically significant.

Results: Four genes (BRCA1, BRCA2, FGFR2 and MA3K1) were associated with CMT ($p<0.005$, $p<0.01$, $p<0.002$, and $p<0.006$ respectively). When benign and malignant cases were analyzed separately a stronger association was seen with the malignant cases for BRCA1 ($p<0.005$) and FGFR2 ($p<0.002$), suggesting that these genes may contribute to malignancy.

Conclusion: This study indicates that FGFR2, MAP3K1, BRCA1 and BRCA2 may contribute to the development of canine mammary tumours in ESS and that canine and human breast cancer may be more similar than previously suspected. The dog may therefore serve as a good model for human breast cancer. A genome-wide association study for CMT is in progress and is expected to identify additional strong risk factors.

Reduced PTEN protein expression and its prognostic implications in canine and feline mammary tumours

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Background: PTEN belongs to the group of gatekeepers tumour suppressor genes and plays a role in neoplastic transformation and progression. We describe its expression and correlation to clinico-pathological parameters in canine and feline mammary carcinomas.

Material and methods: 24 dogs and 17 cats were submitted to a two-years follow-up. PTEN expression evaluation was semiquantitative, based on the cytoplasmic staining intensity and distribution in accordance with previously reported scoring methods in human oncology. The PTEN results were correlated to the presence of lymphatic vessels invasion, lymph node metastases, mitotic index, tumour grading, and overall survival (OS).

Results: In both species, PTEN expression was detected in the cytoplasm of healthy and hyperplastic mammary and stromal cells. In dogs all of the benign and 16 (67%) of malignant tumours were PTEN-positive. Four (24%) feline mammary carcinomas (FMCs)were PTEN-positive. In canine mammary carcinomas (CMCs) there was a significant correlation between IHC loss of PTEN protein expression all the parameters examined, while in FMCs a PTEN loss was correlated to lymphatic invasion. A reduced OS was observed in subjects bearing PTEN-negative tumours.

Conclusions: This is the first report of PTEN protein expression in FMCs and of a correlation between PTEN expression and the clinical outcome in CMCs. PTEN loss of expression occurred in 33% of canine and 76% of feline mammary carcinomas, and was correlated to several markers of malignancy in CMCs. Although a wider study is required, loss of PTEN expression could be considered useful as a prognostic marker also in veterinary oncology.

Protective effect of misoprostol against increased gastrointestinal barrier permeability caused by meloxicam with and without concurrent dexamethasone

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In addition to anti-inflammatory and analgesic properties, cyclooxygenase inhibitors have antitumor effects against some tumors expressing COX-2. Adverse effects due to inhibition of protective prostaglandin production may result in gastrointestinal and renal damage, limiting COX-2 inhibitor use. Corticosteroids have similar adverse gastrointestinal effects. Concurent COX inhibitor and corticosterioid use is contraindicated due to synergistic adverse effects. The synthetic prostaglandin E1 analogue misoprostol is used clinically to combat these advere effects.

The aim of the study was to determine the effect of misoprostol (0,1mg/kg/q8h) on increased gastrointestinal barrier permeability caused by exposure to meloxicam (0,2 mg/kg/q24h) and/or dexamethasone (1mg/q24h 3 days).

Seven healthy beagle dogs had triple sugar gastrointestinal permeability tests performed at intervals during (days 2 and 6) and after (days 1 and 10) various treatments: placebo (saline 3ml PO q24h, 10 days), meloxicam daily (10 days), meloxicam and misoprostol (10 days), meloxicam and dexamethasone (10 days), and meloxicam, dexamethasone and misoprostol (10 days), with a 10+ days recovery period in between. Testing involved peroral administration of isotonic triple sugar solution (100 ml) containing saccharose, lactulose and mannitol and measurement of sugar concentrations in blood samples, obtained two hours later, using thin layer chromathography. Saccharose concentration and lactulose/mannitol index were used as indicators of gastrointestinal permeability.

The test confirmed that meloxicam alone or combined with dexamethasone increased gastrointestinal permeability. Misoprostol significantly (paired T-test, $p<0.05$) reduced the increase in permeability caused by meloxicam and dexamethasone. Misoprostol therefore has a protective effect on gastrointestinal barrier permeability during meloxicam and meloxicam/dexamethasone treatment.

Application of photodynamic therapy in cases of tumor-bearing companion animals.

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Photodynamic therapy (PDT) is a relatively new and auspicious treatment of several human and animal tumors. Tissue damage occurs when a photosensitizer is activated by light of an appropriate wavelength in the presence of oxygen. Nowadays, it is considered an investigational treatment modality in small animal oncology, mainly for companion animals. Preliminary veterinary studies were contemporary with early human clinical trials of PDT, but no well defined treatment protocols have been established for clinical veterinarians up to the present.

PDT treatment is best suited for well localized, small size tumors. At our Faculty of Veterinary Science we are making efforts to introduce PDT for the first time in Hungary into oncology of companion animals affected mainly by squamous cell carcinoma, mast cell tumors, and different types of sarcomas. We are also investigating the use of PDT of larger tumors without or after a surgical reduction.

Case reports will be presented of dogs and cats with different types of cancer treated with 5-aminolevulinic acid (ALA) as a photosensitizer precursor drug. An optimal accumulation of the photosensitizer was usually achieved after approximately 3 hours, then the tumor was illuminated using a 250 W halogen lamp at a wavelength of 590-728 nm and 100 J/cm^2 light fluency.

Although long-term follow up reports are not yet available, early results are promising in several cases.

***In vitro* synergistic antiproliferative effect of Recombinant Feline Interferon-ω with chemotherapy agents on canine and feline mammary tumours and derived cancer stem cells.**

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Interferons are naturally produced cytokines with multiple important biological functions. We have previously shown the *in vitro* antiproliferative activity of recombinant feline interferon-ω (rFeIFN-ω) on feline and canine mammary carcinoma cells (CAT-MT and REM134) and derived cancer stem cells (CSC) with a species-specific, dose dependent and target cell specific action.

In this study we tested the effect of rFeIFN-ω on REM134 and CAT-MT cell lines and derived CSC in association with conventional chemotherapy drugs. Cells were seeded in 96-well plates and incubated at 37 °C at 5% CO_2 for 24 hours. Serial dilutions of doxorubicin, mitoxantrone, vincristine and cyclophosphamide were added together with rFeIFN-ω at a previously calculated IC_{50} for each parent cell line and derived CSC. Plates were then incubated at 37 °C at 5% CO_2 for 72 hours. Viability (percentage of viable cells) was measured by chemoluminescence with Cell Titer-Glo Kit (Promega).

A 1-way ANOVA and Student t tests were used for statistical analysis. Synergistic effect was noticed between rFeIFN-ω and conventional anticancer drugs, in particular antracyclines. rFeIFN-ω may be a useful antineoplastic agent in feline and canine mammary tumours.

COX-2 expression in canine intranasal tumors

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Background:

The expression of cyclooxygenase-2 (COX-2) by neoplasms has been documented. Recently it was shown that COX-2 expression is present in 71-87% of all canine intranasal carcinomas. However, little is known about expression in various subtypes of carcinomas and up to now non-carcinomatous intranasal tumors have not been investigated.

Methods:

Immunohistochemistry for COX-2 expression was performed on formalin fixed material of 113 histopathologically confirmed canine intranasal tumors. At least 20 cases of each of the following types of intranasal tumors were examined: adenocarcinoma (ACA), squamous cell carcinoma (SCC), transitional cell carcinoma (TCC), chondrosarcoma (CSA), olfactory neuroblastoma (ON). Distribution and intensity of COX-2 staining of the neoplastic cells was evaluated by a board certified pathologist (WvB).

Results:

Overall, COX-2 expression could be demonstrated in 73% of all specimens, and 81% of the carcinomas. There were pronounced differences in staining characteristics between the various carcinoma subtypes. 100% of SCCs and 85% of TCCs showed COX-2 expression, but only 61% of ACAs stained positive. ON showed COX-2 expression in 81% of the cases. The lowest expression rate was found in CSAs with only 41%.

Conclusion:

COX-2 expression can be found in all subtypes of canine intranasal tumors but expression is markedly different between various subtypes of neoplasias. Interestingly, a high percentage of COX-2 expression could be demonstrated in ON, which was somewhat unexpected. Based on these results, COX-inhibitors such as non-steroidal anti-inflammatory drugs (NSAIDs) may be a promising treatment option for these types of tumors and should be evaluated in clinical studies.

met oncogene activation qualifies spontaneous canine osteosarcoma as a suitable pre-clinical model of human osteosarcoma

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Background: Spontaneous tumours in companion animals are considered suitable models for human carcinogenesis. Canine osteosarcoma (OSA) showed histopathological and clinical features similar with human osteosarcomas. The *MET* oncogene-encoded tyrosine kinase receptor is aberrantly expressed in human osteosarcoma and is an attractive molecular target for cancer therapy. In this research we studied spontaneous canine osteosarcoma (OSA) as a pre-clinical model for Met targeted therapies.

Methods: Expression and activation of the canine Met receptor was studied with immunohistochemistry in 39 samples of dog osteosarcomas, including 35 primary tumors and 4 metastases. mRNA and total protein were extracted from 6 canine osteosarcoma cells line, from normal cells lines and from primary canine osteoblasts cells lines; ShRNA anti MET using lentiviral vectors and biological in vitro assay on D22 and D17 cells line was carried out.

Results: High level of expression was found in 80% of dog osteosarcoma samples from various breeds. When the Met receptor was over-expressed, its activation was detectable as phosphorylation of critical tyrosine residues. *met* oncogene expression was also detectable in 6/6 canine osteosarcoma cell lines. *met* over-expressing OSA cells showed constitutive receptor activation and elevated spontaneous motility and invasiveness, which was impaired by both Met kinase inhibitor PHA-665752 and *met* specific, stable RNA interference obtained by means of Lentiviral vector driven cell transduction.

Discussion: These data show that *met* over-expression characterizes canine OSA as homologous to the human counterpart and as a suitable model to test *MET* targeted therapies of metastasizing OSA.

^{111}In-pentetreotide scintigraphy in small animal insulinoma: interest of single-photon emission computed tomography (SPECT) and CT image fusion in three cases.

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CT and SPECT with ^{111}In-pentetreotide have been described in dogs for characterisation of insulinomas. Their drawbacks, poor specificity and low prediction of anatomical location respectively, suggest an interest in the fusion of images obtained concomitantly by both techniques. Our goal is to evaluate the feasibility of fusion of SPECT and CT images of canine and feline insulinomas.

Methods: Insulinoma was diagnosed in 2 dogs and one cat. Planar scintigraphy, SPECT, low- and high-resolution (LR/HR) CT were performed after injection of ^{111}In-pentetreotide. SPECT and HR CT images were fused thanks to landmarks of LR CT images.

Results: Ten sites of abnormal uptake were detected by SPECT *vs* 6 by planar scintigraphy. Metastasis were suspected because of their location distant from pancreas for 4 foci. Discrimination between pancreatic and extrapancreatic localization was impossible for 6. One pancreatic lesion was identified by CT in each case. Two hepatic lesions and 3 enlarged lymph nodes were detected. Each site of uptake was superimposed to a CT lesion except for 2. Laparotomy on one dog and the cat confirmed the tumoral infiltration of all the sites designed by superimposed images. Peritoneum area around one of the 2 unfused foci presented similar histopathological description.

Conclusion: This description of SPECT/CT exams confirms the superiority of SPECT over planar imaging for insulinoma characterisation. In these cases, the fusion congruence was observed for all the lesions on CT with a subsequent increased specificity. Besides 8/10 SPECT foci were combined with abnormal CT images offering a gain of localization information.

Expression of *TWIST1*, *ERBB2* and *TP53* genes in normal, hyperplastic and neoplastic cat mammary gland tissues

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TWIST1 is a novel oncogene. Proto-oncogene *ERBB2* overexpression correlates with more aggressive clinicopathologic features and drug resistance in breast cancer. *TP53* is a tumor-suppressor-gene and disruption of its function strongly correlates with tumorigenesis. The aim of this study is to evaluate the feline *TWIST1*, *ERBB2* and *TP53* expression in normal, hyperplastic and neoplastic mammary gland tissues using qRT-PCR.

After RNA extraction/purification, cDNA synthesis was performed. Primers were designed and hybridization probes were selected according to major homology for each transcript. *TWIST1*, *ERBB2* and *TP53* expression was quantified by qRT-PCR in several mammary tissues: 3 normal, 7 carcinomas, 1 benign tumor and 3 hyperplasias. GADPH was used as housekeeping gene.

TWIST1 was downregulated in all carcinomas and in 2/3 hyperplasias and revealed lower expression levels in carcinomas than in benign tumors/hyperplasias. *ERBB2* was downregulated in all tissue samples. *TP53* showed to be upregulated in 4/7 carcinomas and in 3/4 benign lesions.

To our best knowledge, *TWIST1* mRNA quantification in normal and tumoral tissues has never been reported in cats. In humans, when increased in breast cancer cells, it promotes metastatic ability. In this study, benign lesions showed higher *TWIST1* mRNA expression than malignant masses. Regarding *TP53*, high expression levels in 35-45% feline mammary carcinomas (FMC) were found. In our samples, a higher expression (57%) is evidenced. It is reported high *ERBB2* mRNA levels in 6/11 FMC. Our study shows statistically significant lower mRNA levels in all FMC (7/7). This study evidences challenging results, suggesting future directions for further investigations in FMC.

General Abstracts

Immunohistochemical study on p53 expression in feline squamous cell carcinoma using cm1 and pab240 clones.

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Background: P53 is a 53kDa nuclear phosphoprotein known as "guardian of the genome" due to its role to maintain the integrity of the genome and its tumour suppressor action. In cells with loss or mutation of P53, DNA damage does not induce cell cycle arrest or DNA repair, giving rise eventually to malignant tumours. SCC in cats most commonly occurs in the head, abdomen, limbs, perineum and digits. The aim of the present study was to investigate P53 expression in feline squamous cell carcinoma by immunohistochemistry.

Methods: Twenty tissue biopsy samples of feline SCC were classified according to the WHO. Immunohistochemistry was performed on tissue serial sections using Pab240 and CM1 primary antibodies, and then incubated with secondary antibody kit (Envision/DAKO).

Results: The cats had a mean age of 12.72 years and included 1 Persian and 19 DSH cats; 12 were spayed females, 2 were intact females, 4 were castrated males and 2 were intact males. Nuclear p53 immunoreactivity was observed for clones Pab240 and CM1 in 35% (7/20) and 40% (8/20), respectively.

Discussion: Nuclear p53 overexpression was more commonly detected with CM1 clone compared to Pab240. All samples were positive for CM1 and Pab240 clones. In a single case, we observed a positive immunoreactivity for the clone CM1 only. However, it should be mentioned that Pab240 identified only the mutant form of the protein. Immunohistochemical detection of p53 in a proportion of cases may suggest that mutation of the p53 gene may play a role in the pathogenesis of SCCs.

Tavocept™, a novel chemoprotectant drug, potentiates the *in vitro* cytotoxicity of cisplatin against canine osteosarcoma

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Background: Cisplatin is one of the more effective drugs for improving survival time for dogs with osteosarcoma (OSA), but requires lengthy saline diuresis to reduce the risk of nephrotoxicity. Tavocept™ is an investigational drug shown to prevent or mitigate common chemotherapy-related toxicity, as well as enhance *in vitro* cytotoxicity of cisplatin against some human cancers. Tavocept™ can be safely administered to dogs alone and in combination with cisplatin, and permits cisplatin dose escalation far beyond that which would otherwise be clinically tolerable. This study was designed to determine the effect of Tavocept™ against canine OSA cells, as well as to assess for potentiation of cisplatin cytotoxicity.

Methods: Canine OSA cells (D17; ATCC) were treated for one hour with Tavocept™ (1.0 and 10 mM), cisplatin (6.25 or 10 μM), or a combination thereof (Tavocept™ for one hour, wash, then cisplatin for one hour) and allowed to undergo 5 doublings in drug-free media. A sulforhodamine B (SRB) assay was utilized to assess cytotoxicity. Data were averaged from two plates per experiment in two independently conducted experiments.

Results: Tavocept™ alone did not result in cell growth inhibition. Cisplatin alone inhibited cell growth to 75% and 58% of baseline, whereas cisplatin in combination with Tavocept™ inhibited growth to 69% and 52% of baseline, respectively.

Conclusions: Tavocept™ enhances the growth inhibition of canine OSA cells by cisplatin and should be further evaluated in a prospective clinical trial. Applications for canine bladder cancer should also be explored, given the efficacy of cisplatin and piroxicam against this tumor type.

Carbohydrate-based galectin-3 inhibitors enhance the cytotoxicity of doxorubicin against haemangiosarcoma

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Background: Galectin-3 (Gal-3) is a carbohydrate binding protein that enhances tumor growth and metastasis and inhibits apoptosis. The anti-Gal-3 compounds, Lactulosyl-L-Leucine (LL) and modified citrus pectin (MCP) can disrupt these functions by blocking Gal-3 binding and may potentiate the anti-tumor activity of chemotherapeutic agents. We hypothesized that LL and MCP would enhance the cytotoxicity of doxorubicin (DOX) chemotherapy and tested this hypothesis *in vitro* and *in vivo* against hemangiosarcoma (HSA).

Methods: The IC_{50} and IC_{min} for LL, MCP and DOX against a murine HSA cell line (SVR) were assessed by clonogenic growth assay. SVR cells were exposed to increasing concentrations of each compound. DOX was titrated on the background of IC_{min} of each compound in order to determine if LL and MCP enhance its cytotoxicity. For the *in vivo* study, SVR cells were implanted in the flank of nude mice and assessed for tumor response to DOX, MCP and a combination thereof.

Results: All compounds inhibited HSA cell growth and survival in a dose-dependent manner. The IC_{min} for LL and MCP were 200 μM and 0.06%, respectively. At their IC_{min} LL and MCP enhanced DOX cytotoxicity, reducing the IC_{50} of DOX 3.6 fold and 10.7 fold, respectively. There was no observable difference in tumor growth between treatment groups in the *in vivo* study.

Conclusions: We conclude that LL and MCP enhance the *in vitro* cytotoxicity of DOX. Protocol optimization is needed to determine if this phenomenon will bear out in the *in vivo* and clinical settings.

Prognostic markers for canine insulinoma

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Introduction Canine insulinoma is an insulin-producing, pancreatic □-cell tumour of adult dogs, which secretes insulin in an uncontrolled fashion, leading to clinical signs associated with

hypoglycemia. Previously, using canine cDNA microarrays, we were able to group 10 canine insulinomas into two subsets based on their different gene expression profiles. The first group of insulinomas appeared to consist of tumours that were more differentiated and had a less malignant behaviour compared to insulinomas from the other group. We hypothesized that there are prognostic biomarkers amongst the genes that were differently regulated between the two insulinoma subsets.

Materials and Methods For six genes, the expression of which was significantly different between the two insulinoma subsets as shown by Significance Analysis of Microarray (SAM) software, the expression levels were quantified in large insulinoma series (n = 26) by qPCR. The insulinoma samples were collected from 26 dogs with known disease free survival (DFS). To determine whether gene expression levels were correlated to clinical outcome, univariate and multivariate analyses were calculated by the Cox proportional hazard regression model using SPSS software.

Results and discussion Pancreatic lipase (PL) was among the highest up-regulated genes in the well-differentiated group of insulinomas, and PL mRNA expression was correlated to DFS of dogs with insulinomas. Since PL mRNA expression was correlated to PL concentrations of insulinoma homogenates, we propose that serum lipase activity may be used as a prognostic biochemical marker in canine insulinomas. Also, larger tumour size was significantly associated with shorter DFS.

Tumor targeting and treatment possibilities using matching pairs of diagnostic and therapeutic somatostatin analogue radiopeptides

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Introduction. Targeted internal radionuclide therapy is a therapeutic possibility for tumor types overexpressing a specific cell surface receptor. We hereby present this "magical bullet" approach through somatostatin-2a receptor peptide diagnostics and radiotherapy cases. Beta radiation is delivered to the tumor cell environment by small targeting peptides.

Methods. DOTA-T-Octreotide (DOTATOC), a somatostatin 2a receptor ligand was labelled with ^{111}In to study diagnostic possibilities in a mixed breed male dog with insulinoma. In a second study, ^{90}Y as a therapeutic isotope was used to label DOTATOC. Two therapy cycles of 370 MBq and 180 MBq were administered to a mixed breed male dog with insulinoma. Insulin levels, blood enzyme values and tumor size ultrasound were followed up. HYNIC-T-Octreotide, a later developed diagnostic counterpart was labelled with ^{99m}Tc and administered to another mixed breed dog with insulinoma. SPECT imaging and clinical follow-up as well as immunohistopathology was performed.

Results. Tumors in the pancreas were visualized using diagnostic SPECT/scintigraphy imaging in all animals. Radionuclide therapy provided complete remission for more than 2 years.

Conclusions. Somatostatin receptor radiotherapy is a viable option for dog tumors, too. This approach can be further extended to other tumor types over-expressing peptide receptors provided the availability of radiopharmaceuticals.

Survival times in dogs with intracranial tumours following radiation therapy

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Medical records of dogs with intracranial tumours treated with radiotherapy (RT) at the QVSH, Cambridge from 1999 – 2007, were examined retrospectively to determine outcome and survival.

31 dogs were identified with diagnosis of intracranial tumour by MRI, all of which had completed a 5 week escalating course of RT delivered by a 4MV Linear Accelerator to a total dose of 38 Gy (as previously described - Brearley et al, 1999).

Presenting signs were seizures or change in mentation with circling and ataxia also being common. The mean age was 7.7 years (range 3 – 12 yrs), with no gender bias. Boxers were over represented (14/31). On the basis of site and MRI findings the tumours were classified as glioma (n=9), meningioma (n = 12) and pituitary macroadenoma (n=7).

All dogs initially responded well to RT, some dogs presenting with seizures needed maintenance therapy with phenobarbitone. Two dogs were still alive at the time of analysis (censored), 7 dogs were lost to follow up (excluded from the survival analysis), the remainder were euthanased due to gradual recurrence of neurological signs. The incidence of adverse reactions to the RT was low. The median survival was 333.5 days, mean 414.3 days (95% CI: 260 – 568 days). Dogs with meningioma showed the most favourable outcome with a median survival 480 days versus glioma, 306 days and pituitary macroadenoma 200 days.

The results of this study support previous reports that hypofractionated RT can provide good palliation for intracranial tumours with minimal toxicity.

(Brearley MJ, Jeffrey ND, Phillips SM et al, JVIM 1999: 13: 408 – 412)

Combination vinblastine, lomustine, and prednisone chemotherapy for high grade II and III canine cutaneous mast cell tumors.

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Background: Grade III mast cell tumours carry a guarded prognosis with reported survivals between 91-280 days with surgery alone. The purpose of this study was to examine a group of dogs with high stage, high grade mast cell tumours, treated with a combination of vinblastine, lomustine and prednisone.

Methods: 17 dogs with grade III tumours and/or metastatic grade II mast cell tumours were were treated with a protocol consisting of 4 x 21 day cycles of alternating vinblastine (2 mg/m^2) day 1, and lomustine (60 mg/m^2) day 11, with a tapering dose of prednisone. Staging for all dogs included CBC, chemistries, thoracic radiographs, abdominal U/S, aspiration of spleen, liver, and enlarged lymph nodes, buffy coat and/or bone marrow examination. Toxicity, and survival were evaluated, and statistical significance for prognostic variables was set at P-value less than 0.05.

Results: At initial presentation WHO staging was II (n=3), III (n=6) and IV (n=8). In 10/17 patients U/S findings did not correlate with cytological findings. Toxicity included: neutropenia grade 4(3x), grade 1(3x), thrombocytopenia grade 1(3x), vomiting grade 1(4x), and diarrhea grade 1(2x). Eight/17 dogs completed the protocol, 3 had drugs discontinued, 6 stopped due to disease progression. Median survival time was 276 days (range 34-367). Poor prognostic indicators for survival included stage IV (median 85 vs. 338 days, P=0.0012), splenic metastasis (median 100 vs. 291 days, P= 0.0012), and inappetance (median 34 days vs.276 days, P=0.0018).

Conclusion: This protocol was well tolerated and appeared to offer survival prolongation with high grade, high stage mast cell tumours.

The VELCAP-C protocol in feline lymphoma: an advisable treatment option?

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Lymphosarcoma, the most common haematopoietic tumour in cats, showed over the last decade an overall increasing incidence, especially in the intestinal form, despite a significant decrease in feline leukemia virus associated lymphomas.

For systemic feline lymphoma chemotherapy is considered to be the most effective therapy. We treated twenty-eight cats with the VELCAP-C protocol, a 6-month protocol consisting of the drugs vincristine, L-asparaginase, cyclophosphamide, doxorubicin and prednisolone. The distribution of anatomic type of the lymposarcoma was thirteen alimentary, five abdominal, seven extranodal, one mediastinal and two mixed. Retrospectively we evaluated toxicosis, response to therapy, and survival time. Median survival time was 59 days (range 4 – 914 days) and 18% of the patients completed the full protocol, which was generally well tolerated. The cats which completed the protocol showed a higher median survival time with 234 days (196 – 823 days). The other cats were euthanized due to clinical deterioration caused by none responding to the chemotherapy.

Our study is in accordance with the surviving time results observed in Tufts, where 61 cats after receiving the VELCAP-C protocol showed a median survival time of 62 days. Interestingly, the use of this multi agent protocol resulted in inferior survival data compared to the older COP protocol producing median survival times up to 9 months. Showing no real advantage it should be considered changing the VELCAP-C protocol for more promising ones in the future treatment of lymphoma cats.

Successful use of aciclovir in equine sarcoid therapy

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Background: Equine sarcoids are bovine papillomavirus(BPV)-induced skin tumours which affect up to 10% of the worldwide equid population. To date, there is no universally effective therapy to cure this common disease. Based on the anecdotally reported eradication of a sarcoid by topical use of Zovirax® cream, we investigated the curative potential of its anti-viral compound, aciclovir, in a cohort of 31 sarcoid-affected horses

Methodology:, From 2006 to 2008, 30 BPV-1-infected horses bearing single or multiple smaller lesions of various type were treated by daily topical application of aciclovir-5% ointment onto lesions for a period of three weeks to six month. In one case, aciclovir treatment was combined with intralesional cidofovir injections. Tumour sizes were evaluated and photo-documented on day 0 and then in two weeks-intervals for up to 6 months.

Results: All sarcoids responded to aciclovir treatment and no adverse effects were observed. Complete tumour regression was observed for 66% of sarcoids, with no recurrence reported so far. Incomplete remission was obtained for 34% of sarcoids that were initially thicker than full-responding tumours.

Conclusion: Aciclovir is an antiviral designed to interrupt herpesvirus replication. To this aim, aciclovir needs to be activated by triple phosphorylation which is achieved by cellular und viral thymidine kinase. Since papillomaviruses do not code for this enzyme, the mode by which aciclovir acts on BPV-induced sarcoids remains unclear. Yet, aciclovir-5% ointment shaped up as inexpensive and non-irritating substance that has been well tolerated and proven effective in the eradication of sarcoids of minor thickness.

BPV-1 VLP Vaccination Phase I Trial in Horses

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Introduction: Equine Sarcoids are benign yet locally invasive skin tumors which affect horses and all other equids. Bovine papillomavirus type 1 (BPV-1) and, to a much lesser extent, type -2 (BPV-2) are accepted as the main causative agents for equine sarcoids. Sarcoids persist, are highly therapy resistant and tend to recur following treatment. As a prophylaxis against this disease, a BPV-1 L1 virus-like particle (VLP) vaccine was evaluated for bio-safety and immunogenicity.

Methods: BPV-1 L1 VLPs were generated in a baculovirus system. Horses which had tested negative for BPV-1/-2 antibodies were immunised. Three groups each comprising four animals were injected three times (on days 0 after 2 weeks and after 6 months) with 50, 100 or 150 µg of BPV-1 VLP plus adjuvant (AlOH 500 µg/dose), respectively. A control group consisting of three horses received adjuvant only. The horses were monitored daily to record possible side effects. Blood was collected before and two weeks after each immunisation. Serum antibody titres were determined by BPV-1 pseudovirion (PsV) neutralisation assay.

Results: No unacceptable side effects were observed. Two weeks after the 6 month booster injection 12/12 of the horses had raised neutralising antibody titres of a minimum of 1:1600, whereas no titres were measured in the control group.

Conclusions and Clinical Significance: Horses vaccinated three times with 50-150µg of BPV-1 VLP produced neutralising antibodies at titres, which correlate with protection against infection in animals and man. Effectivity of protection will be tested in a phase II challenge study in the near future.

Cyclooxygenase-2 overexpression correlates with vascular endothelial growth factor expression and tumour angiogenesis in canine mammary cancer.

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Background: Angiogenesis is a key prerequisite for the successful establishment, growth, and dissemination of tumors. Vascular endothelial growth factor (VEGF) has a potent angiogenic activity and cyclooxygenase-2 (COX-2) promotes angiogenesis by modulated production of angiogenic factors including VEGF. The present study was designed to investigate the possible roles of COX-2 and VEGF in canine mammary cancer angiogenesis.

Methods: In this study, we performed an immunohistochemical investigation of COX-2 and VEGF expression in 70 tumours (28 benign and 42 malignant). To assess tumor angiogenesis, micro-vessel density (MVD) was determined by CD31 immunohistochemical staining.

Results: Cox-2 ($p<0,001$) and VEGF ($p<0,001$) expression were significantly higher in malignant tumours with respect benign neoplasms. Malignant tumours had also a higher MVD than benign counterparts ($p<0,001$). In malignant tumours the expression of COX-2 was positively correlated with expression of VEGF and MVD ($p=0,008$ and $p= 0,026$, respectively). The mean MVD value of VEGF positive tumours was significantly higher than that of VEGF negative tumours ($p<0,001$). The mean MVD value of COX-2 positive tumours was significantly higher than that of COX-2 negative tumours ($p=0,026$). The mean value of MVD in tumours positive for COX-2 and VEGF was significantly higher than that in tumours considered negative for both ($p<0,001$). Additionally, Cox-2 and VEGF overexpression were associated with infiltrative growth; mitotic index and nuclear grade but not with tumour size. VEGF was also related with tumour necrosis.

Conclusions: The present findings suggest that overexpression of COX-2 may induce the expression of VEGF, increase angiogenesis, and enhance tumour growth.

MRI evaluation of the canine mammary tumours

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Background
The aim of this study was to apply static and dynamic magnetic resonance imaging (MRI) sequences adapted from the human medicine in the examination of canine mammary glands and its tumours.

Methods
Static (T1- and T2-weighted spin echo (SE), short Tau inversion recovery (STIR) on 28 and dynamic (T1-weighted gradient echo (GE)) contrast enhanced (DCE) MRI sequences in coronal, sagittal and transversal planes were performed on 15 dogs of different breeds.

Results
The static MR technique is the most detailed imaging modality for differentiating the tissue types in the substance of the mammary gland. The MRI findings were in close relationship with the histological result. Using the GE DCE sequence the morphological patterns as well as the kinetic parameters proved to be valuable parameters about the mammary tumours and initial information was obtained on the contrast enhancing properties, which are necessary factors during in vivo staging and in the prognostic work.

Conclusion
With static MR sequences the mammary gland and the neighbouring anatomical structures could be examined in great detail with optimal contrast conditions. The contrast accumulation methods and curves prepared for humans are of great promise in veterinary use but need to be adapted and a sensitive protocol should be completed, which makes the detection of canine mammary tumours possible. In this study the adapted sequences proved to be applicable for screening mammary tumours, but more research is needed, as at present we cannot tell if a tumour is benign or malignant on the basis of MR imaging.

Long term follow-up and predictors of survival in dogs with mammary inflammatory carcinoma: a retrospective analysis of 43 cases.

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Background. Canine mammary inflammatory carcinoma (IC) has a grave prognosis. Prognostic factors and treatment data have not been studied extensively.

Objective. To describe clinical characteristics, treatment and outcome of dogs with IC, and identify patient-, tumor-, and treatment-related predictors of overall survival.

Methods. Records of dogs with a clinical diagnosis of IC showing pathological evidence of dermal lymphatic invasion were reviewed. Data on clinical staging, type of treatment, toxicity, response, and survival time were retrieved.

Result. 43 client-owned dogs were enrolled. Primary IC accounted for 60.5% of cases, whereas secondary IC represented 39.5% of cases. The overall metastatic rate at presentation was 86.0%, with 81.4% of dogs showing distant metastasis. Six of 29 dogs had an abnormal coagulation profile. Sixteen dogs never received treatment, 24 received medical treatment only, 2 underwent surgical excision and medical treatment, and one underwent surgery only. Forty-one of 43 dogs experienced progressive disease, and 2 dogs were stable, never reaching disease-free status. Mean survival for all patients was 60 days (range, 1 to 300 days). The presence of coagulopathies decreased survival of approximately 70% (OR: 0.28, CI 95%: 0.10-0.80, $p < 0.05$) and the use of medical therapy increased the chance of survival of more than twofold (OR: 2.54, CI 95%: 1.27-5.08, $p < 0.01$).

Conclusion. IC is biologically aggressive and has a guarded prognosis. Complete staging at presentation, including coagulation profile, is mandatory. Results suggest that medical treatment may improve outcome, thereby supporting its use in dogs with IC.

HER-2 Expression in canine healthy, dysplastic, and neoplastic mammary tissues

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Background: The protooncogene HER-2 encodes a transmembrane receptor protein with tyrosine-kinase activity. Previous studies have shown that HER-2 overexpression is present in the canine tumours; however, possible analogies between protein expression in the canine and human species and the correlation of HER-2 with the prognosis are still controversial.

Methods: Twenty four canine mammary carcinomas, 11 mammary adenomas, and healthy and hyperplastic tissues at the periphery of the tumors were evaluated by immunohistochemistry (IHC) for HER-2 expression. Sections from four HER-2 overexpressing human mammary carcinomas were included in the study. IHC was performed with the Herceptest® Dako and by using the same primary antibody and a different detection system. HER-2 expression was evaluated according to the Herceptest scoring guidelines and according to previously published scoring systems. HER-2 protein expression was also correlated to tumour histologic type, grading, mitotic index, lymphatic invasion, and overall survival.

Results: HER-2 overexpression was detected in 3/11 adenomas and 4/24 carcinomas. Normal and hyperplastic mammary tissues examined were often positive. In carcinomas, HER-2 positive status did not correlate with the parameters examined. A positive correlation was observed between tumour HER-2 positive status and presence of HER-2 overexpression in hyperplastic and normal mammary tissues surrounding the tumours.

Conclusions: The results revealed that HER-2 overexpression quantification may be affected by the method and the scoring system used. Furthermore, in contrast with human breast cancer HER-2 overexpression in the canine species does not seem to identify a subgroup of tumours with a poor prognosis.

Environmental dust exposure and lung cancer in dogs: evidence for a link.

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Background. Primary lung cancer in dogs has been putatively related to urban living and air pollution on epidemiologic basis, but a causative association has not been definitely shown. Furthermore, the relationship between the deposition of black dust matter in lungs (anthracosis) and primary pulmonary carcinoma has not been investigated in dogs.

Methods. Amount, type and birefringence of pulmonary anthracosis were histologically assessed in a retrospective study by examining 35 dogs with primary epithelial lung cancer and 160 controls; the odd ratio (OR) was calculated for dogs with primary lung cancer. The same factors were analyzed to identify an association between type of lung tumor, histological grade, and TNM stage .

Results. Papillary adenocarcinoma was most commonly diagnosed (45.7%). The majority of tumors was of histological grade 2, and the lung cancer was more often localized (clinical stage I). An increased risk for lung cancer was observed in dogs with higher amounts of anthracosis (OR: 2.20, CI 95%: 1.27-3.81; $p < 0.01$). Type of anthracosis and its birefringence were not related to lung cancer. Tumor type, histological grade, and TNM stage were not associated with amount, type and birefringence of anthracosis.

Conclusions and Clinical Relevance. Anthracosis due to inhalation of polluted air contributes to lung cancer development in dogs. Further studies are required to characterize the specific environmental contaminants responsible for lung carcinogenesis in this species.

Expression of Serotonin and its 5-HT1A Receptor in Canine Cutaneous Mast Cell Tumours

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Background: Mast cells of a number of different animal species have been reported to contain serotonin (5-hydroxytryptamine; 5-HT), a monoamine capable of numerous and complex actions, which may include an impact on tumour growth. Few studies on serotonin expression on normal or neoplastic mast cells have been performed in dogs, and such studies have essentially shown negative results.

Methods: In the present study, canine cutaneous mast cell tumours of Patnaik histological grades I to III were investigated for immunohistochemical expression of serotonin and its receptor 5-HT1A. Tumours were examined for both the proportion of positively staining cells and the intensity of staining in individual cells. In order to further demonstrate the presence of serotonin and its receptor in mast cells, a double immunofluorescens technique was applied.

Results: Serotonin and the 5-HT1A receptor were expressed by non-neoplastic dermal mast cells and by neoplastic mast cells. More neoplastic mast cells expressed serotonin than the 5-HT1A receptor. Poorly differentiated tumour expressed fewer of both molecules. The better differentiated mast cells at the periphery of canine cutaneous mast cell tumours had more consistent serotonin expression.

Conclusions: This study suggests a possible role for serotonin and its receptor for the differentiation of canine mast cell tumours and for the paraneoplastic and other clinical effects of mast cell tumours in dogs.

Combination vinblastine, lomustine, and prednisone chemotherapy for high grade

Efficacy and safety in an open label single arm multi center phase III trial of a new formulation of paclitaxel (Paccal® Vet) in dogs with mast cell tumours grade II and III.

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Introduction: Paclitaxel (Taxol®) requires extensive premedication and slow infusion (3-24h) due to side effects caused by the solvent Cremophor EL®. The purpose of this phase III study was to determine the toxicity (VCOG grading system), quality of life (according to a performance status score - PSS) and efficacy of a new micellar, nanoparticular, water-soluble and Cremophor-free formulation of paclitaxel (Paccal® Vet) in dogs with refractory/recurrent mast cell tumours (MCT) grade II/III.

Methods: Paclitaxel (Oasmia Pharmaceuticals, Uppsala, Sweden), dissolved in Ringer-Acetate, was given as a 30 min IV infusion of 150 mg/m^2 with subsequent dose reduction if toxicity was observed (range 150-135 mg/m^2). Treatment was repeated every 21 days for three cycles or until disease progression. No premedication, besides occasional sedation, was administered. Eight study centers participated, including six Swedish and one in Germany and Austria respectively.

Results: Twenty-nine dogs received paclitaxel (73 doses). Grade 3/4 neutropenia occurred in 23.7 and 27.1% respectively. All other grade >2 adverse events occurred in a frequency of less than 10%. One dog with elevated ALT at inclusion had a grade 3 ALT. No drug-related hypersensitivity occurred. Twenty-three dogs received three cycles and the overall response (complete and partial) was 69.5%. The progression free survival at abstract submission was 235 days. The majority kept a normal PSS and many improved as treatment with paclitaxel generated a tumour response.

Conclusions: This is the first successful trial of a paclitaxel formulation in canine MCT. The tumour response and controllable side-effects call for a randomised controlled multicenter trial.

Boxers Do Not Have an Increased Incidence of Genomic Mutations in *c-kit* Exons 11 and 12

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Background: Mutations in *c-kit* have been associated with several types of neoplasia. Previous studies evaluating c-kit in MCTs have demonstrated the majority of the mutations occur in the 11th and 12th exons. Certain breeds of dogs have a higher incidence of MCT development, suggesting these breeds have a genetic predisposition to MCTs. We hypothesized that boxers would have mutations in the 11th and 12th exons of c-kit at the genomic level when compared with breeds with a low incidence of MCTs.

Materials and Methods: 35 blood samples were collected from dog breeds with a low incidence of MCT: toy breeds (14), Border Collies (4), Shetland Sheepdogs (5), Poodles (7), Arctic breeds (4), and Australian Shepherds (1). 37 blood samples were collected from Boxers. DNA was extracted using the Quiagen midi-kit. Exons 11 and 12 and intron 11 were amplified with PCR. PCR products were directly sequenced in the non-disposed dogs. With Boxers, PCR products were subcloned using TopoClone (Invitrogen) and 6 clones sequenced. All sequencing results were compared to the published canine c-kit sequence with BLAST.

Results: In the non-disposed dogs, one single nucleotide polymorphism was found in intron 11. In the boxers, only changes in single nucleotides were found and one possible insertion mutation. This differs from the most common mutation found in MCTs--tandem duplications. One SNP was consistently found at position 84.

Conclusion: Inherited mutations in boxers at exons 11 and 12 of *c-kit* are unlikely to be the cause of MCTs in this breed.

Masitinib reduces the onset of metastasis and improves long-term survival in dogs with measurable grade 2 and grade 3 mast cell tumours.

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We evaluated masitinib in multicenter, randomized, placebo-controlled, double-blind study. 202 dogs having recurrent or non-resectable grade 2/3 cutaneous MCT without lymph node or visceral metastases received either masitinib (12.5 mg/kg/day p*er os*) or placebo. Endpoints were tumour response/progression (WHO criteria) and overall survival (OS).

Masitinib significantly reduced lymph node and visceral metastases. Only 3.7% of dogs under masitinib developed lymph node or visceral metastases (*versus* 17.1% of dogs under placebo; $p<0.001$).

Masitinib was especially efficient on non-resectable tumours. At 24-months, 11.8% of dogs remained in complete remission, no dog in the placebo group. Masitinib also improved survival, at 12-months (61.3% *versus* 37.5% under placebo, $p=0.041$) and at 24-months (36.4% *versus* 15.0%) and almost doubled the survival time with a median survival of 617 days (*versus* 322 days).

Masitinib was also particularly efficient as a first-line therapy. At 12-months, 39.6% of dogs under masitinib had controlled disease (*versus* no dogs under placebo, $p=0.002$) and 14.6% were in complete remission. Moreover, at 24-months, 14.3% of dogs remained in complete remission. Masitinib significantly increased survival rate at 12-months (67.9% versus 37.5% under placebo, $p=0.042$) and 24-months (48.9% versus 14.3% under placebo, $p=0.03$) and doubled survival time with a median survival of 823 days (*versus* 322 days, $p=0.021$).

In conclusion, masitinib significantly reduced the development of lymph node and visceral metastases, induced a long-lasting control of tumour progression and was curative (complete remission at 24-months) in a subset of patients. When masitinib was used as a first line treatment and/or on non-resectable tumours, it was particularly efficient and significantly improved long-term survival.

Assessment of response to the treatment with masitinib (MASIVET®) of chemo-resistant, grade 2 and 3 metastatic canine cutaneous mast cell tumours: report of four cases.

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Introduction C-Kit receptor with the ligand stem cell factor is involved in cell division, differentiation and survival of canine mast cell tumours (MCT). Thirty percent of canine MCT display a mutation in exon 8, 9, 11 or 17 of the c-Kit proto-oncogene. Masitinib is a novel tyrosine kinase inhibitor, targeting both c-Kit receptor (mutated and wild type) and PDGFRβ, FGFR3 and FAK pathway.

Material and methods Four dogs with chemotherapy resistant, metastasized, grade 2 and 3 cutaneous MCT were treated with masitinib (10-12.5 mg/kg/day p.o.). Prior to the treatment with masitinib, these dogs received several cycles of prednisolone, vinblastin, lomustin and radiation therapy. All MCT became resistant to these treatment modalities. In fact, previous to treatment with masitinib the quality of life of all dogs warranted euthanasia. The mutation status of c-Kit has been analyzed in three of the four dogs.

Results In one dog a deletion mutation in c-Kit (exon 11) was detected; in the other two no mutation was present. Masitinib induced CR in two dogs (one had the c-Kit mutation), with a notable response within seven days after start of therapy. PR and SD were obtained in the dogs with wild-type c-Kit. Treatment duration so far is 45 days. Overall tolerability of masitinib was good, and no side effects were noticed in this small study.

Conclusion Masitinib seems an efficient drug to induce remission for chemotherapy resistant grade 2 and 3 MCT. A longer follow up is necessary to evaluate whether the remissions are durable.

Oncothermia –theory and biophysical principles

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Objective: Hyperthermia (HT), despite its long history, could not be accepted in oncology, because of its many controversial results and pure control. Problem was missing technique of the selective deep heating, making various side- and contra effects. Our aim is to present a new technique, oncothermia (OT), which could solve many acute problems with modern facilities.

Material and Methods: OT is capacitive coupled heating, using the constrained conductive 13.56 MHz radiofrequency (RF). The time-fractal modulated RF-current flows through the patient's body between two electrodes. Due to the higher metabolic rate of the malignant cells their ionic activity is larger; in consequence the conductance of the volume is also higher.

Results: The above electronic solution could produce definite difference by the heating of the extra- and intracellular electrolyte. It constrains heat-flow through the membrane, modifying its stability and all the reaction in which it is involved. The conductance differences automatically selects between the cells. The thermal- and electric-gradients, (non-equilibrium condition) govern numerous effects, like the higher membrane permeability, larger intracellular pressure, imbalance of the ion-exchange, change of adherent connections, induce apoptotic signals, helps the expression HSP70 on the cell-membrane, etc. These biophysical effects could lead to intensive distortion of malignant tissue. The method could be combined with any other oncotherapies. It has very few side-effects and rare contraindications.

Conclusion: OT is scientifically based therapy, applied in the human oncology practice. OT is feasible to go over the difficulties of the selective deep-heating, and could be a candidate of modern veterinary oncology.

Oncothermia basic research - results of the *in vivo* studies

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Objective: Discussions about the role of temperature effects has a long history in the hyperthermia applications in oncology. Debate is intensive for electromagnetic heating in tumor. Our objective is to study the temperature independent factors in modulated capacitive coupled, tissue-specific, radiofrequency heating method, oncothermia, (OT).

Material and Methods: Nude mice have been xenografted with HT29 human colorectal carcinoma cells. 28 mice in 4 groups each with 7 animals and each animal with 2 tumors (totally 56 tumors) were included in the present study: group (1) untreated control ; (2) treated with conventional hyperthermia (HT) at 42° C; (3) treated with OT at identical 42° C; (4) treated with OT at 38° C (by intensive cooling the tumor). 24 hours after a single treatment animals were sacrificed and the tumor cross-sections were processed with standard HE staining and were studied by digital microscopy using morphometric software for measuring the respective relative amount of destroyed tumor cells.

Results: Effect of OT established a double effect as a synergy between the purely thermal (temperature dependent) and non-thermal (not directly temperature dependent) effects. We had shown the solely thermal enhancement ratio (TER) of the cell-killing is 2.9. The field enhancement ratio (FER) at constant temperature of 42°C was measured as 3.2. Their complex application increased significantly the therapeutic enhancement to 9.4. We observed significant synergy between the purely temperature dependent and thermally induced but not directly temperature dependent effects.

Conclusion: The oncothermia is about three times more effective than the applied heat alone.

First veterinary clinical results of electromagnetic hyperthermia (Oncothermia®) as a single modality and in combination with fractionated Cobalt irradiation

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Objective: Advantageous thermal and induced non-thermal effects of electromagnetic hypertermia (EHT) in oncology is known for decades. This ever-developing technique has been utilized in human oncology for a long time but surprisingly no veterinary clinical data are available therefore.

Material and Methods: We applied EHT with capacitive coupled modulated 13.56MHz radiofrequency method (oncothermia OT). OT was provided as a single treatment in 6 cases, and in a combination with fractionated Cobalt irradiation in 18 cases. Superficially located skin tumors (mastocytoma GrII, III 7 cases, 2 histiocytomas), 5 malignant oral tumors (3 melanomas, 1 carcinoma), 3 osteosarcomas, 2 nasal cavity adenocarcinoma and 1 insulinoma were treated in dog and 2 feline mammary carcinoma and 3 soft tissue sarcoma cases were enrolled to the study.

Results: Single OT resulted significant tumor size decrease 2 of 6 cases, 3 stable disease and 1 progressive disease. Cobalt irradiation followed by OT resulted 3/18 tumor-free status, 12/18 partial remissions, 2/18 stable disease, and progressive disease in 1 case. Side effects, erythema (2 cases), necroses (2 cases), occurred at the learning phase of technique, later on we could prevent this side effects with the constant superficial and deep temperature control in our patients.

Conclusion: We concluded that local OT could be a useful tool as a single antitumoral modality but even more clinical utilities could be reached in a combination with radiotherapy (maybe with chemotherapy as well) by the local increase of the blood-perfusion. Further clinical studies needed to implement this novel technique into veterinary oncological practice.

Approach to identify multidrug resistance from the genes through the proteins, their function and their role in treatment of canine lymphoma

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Gene. MDR1 (Pgp), mrp1, mrp2 and BCRP protein mRNA expression was identified in lymph nodes. Groups: control dogs (n=7), dogs with primary lymphoma (n=43), lymphoma with septic side effects (n=3), patients with relapse (n=7). MDR1 was decreased in all patient groups compared to controls. MDR1 was increased in relapsed group compared to primary lymphoma group. Mrp1 was decreased in "septic" group compared to controls. BCRP was decreased in relapsed and "septic" group compared to controls. MDR1 inversely correlated with the relapse free period (RFP) and MDR1 such as BCRP inversely correlated with the overall survival time (OST). MDR1 was correlated with the age.

Protein. Immunohistochemistry analysis was performed to identify MDR1 or Pgp in lymph node sections of 27 animals with lymphoma. OST and RFP of MDR1 positives were 7.5 and 3.5 months, respectively and MDR1 negatives 18 and 10 months, respectively. MDR1 inversely correlated with the OST and RFP and higher MDR1 was detected in T-cell type lymphomas than in B-cell types.

Function. Membrane transporter function of separated blood lymphoid cells of 9 dogs with lymphoma receiving chemotherapy and control (n=6) dogs was analysed. One part of lymphocytes was incubated with calcein without inhibitors. Second was inhibited by verapamil (inhibits Pgp, mrp1). Third was inhibited by MK-571 (inhibits mrp1). FACS method was used. Expressing fluorescent activity of the cells MAF- (multidrug resistance activity factor-) values were calculated. Summarized MAF of the patients and the efflux pump function of mrp1 respectively ($p<0,01$, $p<0,05$) increased significantly compared to the control dogs.

Surgical Oncology Abstracts

Anatomical definitions of compartments and surgical planes – dissections and CT scans

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Complete surgical removal of localized cancer cures more cancer patients than any other form of treatment (1). Margins' "Issue" is a continuing dilemma in human and veterinary surgical oncology and is challenged by new research results with trends toward less aggressiveness and adapted to specific tumor types and their anatomical locations. Rational approach in terms of "biologic" rather than »geometric« approach, combining with the expected growth behavior of invasion and spread for the given tumor type mostly gives better results (2)

Regardless of all these variability some basic rules remain in value: invasive cancer should not be peeled out, shelled out, enucleated, or curetted. Many tumors are surrounded by pseudocapsule, composed of compressed and viable tumor cells which should not be entered but remain enveloped with protective tissues barriers.

Happily, human and animal body is composed of tissues with greater resistance to invasion of aggressive tumor cells and their products compared to another. The common denominator of these tissues is "density", given by greater content of collagen, produced by fibrocytes.

In general, tumors initially tend to extend along vessels, nerves, and natural tissue planes. The most effective biologic barriers for tumors invasion are collagen rich, vascular poor tissues, and include fascias, tendons, ligaments, and cartilage. Fat, subcutaneous tissue, muscle and parenchymal tissues offer less resistance to the spread of tumor cells and their invasion-promoting substances. Whenever possible, tumors are removed completely enclosed or embedded in an envelope of normal tissue; usually this space is surrounded by fascia.

The connective tissue that separates and surrounds the more obviously important structures is generically known as fascia, a term of rather elastic usage; many of its larger accumulations, particularly those of a sheet like nature, have specific names and will be shown and discussed during presentation.

Superficial fascia, subcutis is a loose, areolar tissue extensively spread bellow the skin,. This area is also a location of subdermal vascular plexus in a dog and cat in regions lacking cutaneous muscle, i.e. legs as well as well as location of the cutaneous muscle of the trunk. This structure is worth paying attention to as it is enclosed by a thin fibrous layer, having barrier properties. It varies in relative thickness and extent but generally

covers the neck and trunk. It may serve as a barrier plane for tumors lying above this structure.

Similar tissue as superficial fascia surrounds many deeper organs, vascular, nervous structures or muscles. It is also principal site for storage of fat.

The deep fascia, what surgeon recognises as »fascia« is generally organised into much tougher fibrous sheets. This layer beneath the superficial fascia extends over most of the body and fuses to bony prominences. In many places it detaches septa that penetrate between muscles, enclosing them individually or in groups.

Sometimes the periosteum, the fibrous covering of the bones, participates in outlining the enclosures. Since dense fascia is relatively impermeable, it determines in certain degree the direction of growth of tumors.

Radioluceny - density of skin, superficial fascia with fat storage and cutaneous muscle included, deep dense fascia, muscles and other structures offer good contrasts for CT scanning, delineating growth depth of tumors and providing guidelines for planning of dissection.

This is the reason why some knowledge of fascias and surrounding structures, their x-ray contrast abilities and their crucial properties of providing barrier for tumors as well as cleavage planes, which allow relatively bloodless access to deeper parts during surgery, is important for surgical oncologist.

References:

1. Withrow SJ, Vail DM: Surgical oncology; in Withrow and Mac Ewen's Small Animal Clinical Oncology, Fourth Edition, Saunders, Elsevier: 157-162, 2007
2. Gilson SD: Surgical oncology; in The Veterinary Clinic of North America, Small Animal Practice, Volume 25, Number 1, Saunders: 1995
3. Dyce KM, Sack WO, Wensing CJG: Textbook of Veterinary Anatomy, Saunders, 1996

Soft tissue sarcomas: how to manage them in relation to margins

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Soft tissue sarcomas (STS) represent 14–17% of all skin and subcutaneous tumours in dogs.[1] STS include a heterogeneous group of malignant tumours characterized by a similar behaviour. These tumours may recur locally after conservative surgery and have a low metastatic rate. The most common histological types in dogs include fibrosarcoma, peripheral nerve sheath tumour (schwannoma, neurofibrosarcoma), malignant fibrous histiocytoma, and hemangiopericytoma.[2,3] Other sarcomas including hemangiosarcoma, osteosarcoma, synovial sarcoma, chondrosarcoma, and lymphangiosarcoma are usually excluded because of a more aggressive local behaviour and a higher metastatic rate.[4,5]

Surgery is the treatment of choice for most STS. The surgical approach has been classified according to the extent of the wound margins as intracapsular (when the tumour is surgically penetrated, also defined as debulking); marginal (when the tumour is excised just outside, or at the pseudocapsule); wide (when a portion of normal tissue is left around the tumour); and radical (when an entire anatomic segment is removed, i.e., amputation).[4] Dogs with low-grade STS are less likely to develop metastases.[4] Based on the evaluation of margins, wide surgical excision with margins of 2–3 cm is recommended [5,6] to decrease the risk of local recurrence.

Marginal and incomplete surgery is usually associated with a higher risk of local recurrence (26–60%),[8,9] and dogs with incomplete margins are 10.5 times more likely to develop local recurrence than dogs with complete margins.[4] Adjunctive therapies such as radiotherapy,[8,10–12] surgical re-excision,[13] and local chemotherapy (OPLA-Pt sponges)[14] have been applied to tumors marginally or incompletely resected, to decrease the likelihood of recurrence. Radiotherapy alone or in combination with hyperthermia has been used as a palliative treatment for non-resectable STS.[15,16]

In a recent paper, results of marginal excision alone (without any other adjunctive therapy) of low-grade STS of the extremities (at, or distal to, the stifle and elbow joints) in 35 dogs have been published.[17] Authors concluded that well-differentiated low-grade spindle cell sarcomas located at or distal to the elbow and stifle joints can be excised marginally (<1–3 cm margin or not including a fascial plane in the deep margin) based on the low local recurrence rate recorded. Histopathologic margins were dirty (12 dogs), clean but close (12), and clean (11). Follow-up after surgery ranged from 210 to 2202 days. Local recurrence and metastatic rates were 10.8% and 0%, respectively. Median DFI and survival time were not reached, because <50% of dogs died of disease-related events. Mean DFI and mean survival time were 697.8 days (95% CI: 559.7–836 days) and 703.5 days (95% CI: 566.6–840.5 days), respectively. There were no significant differences among survival functions stratified by histological margins.[17]

Margins of 1–3 cm are reported to be acceptable for grade I and II STS, especially when the fascia is included in the deep margin.[18] Reported prognostic factors for local recurrence and risk of metastatic disease, other than surgical margin, include tumour grade.[1,4,5,17,18,19] Metastatic rate for STS is usually low, ranging from 0% to 17%.[4,12,13, 18] Prognosis of grade III STS is worse after en bloc resection and adjuvant chemotherapy (doxorubicin); in 39 dogs that were treated, the local recurrence rate was 23%, the metastatic rate was 44%, the median disease-free was 724 days, and median survival time was 856 days; no beneficial effect of doxorubicin was shown. [19]

References

1. Liptak JM, Forrest LJ: Soft tissue sarcomas, in Withrow SJ, Vail DM (eds): Withrow & MacEwen's: Small Animal Clinical Oncology (ed 4). St Louis,MO, Saunders, 2007, pp
425–454
2. Mauldin GN: Soft tissue sarcomas. Vet Clin North Am Small Anim Pract 27:139–148, 1997
3. Williamson MM: Cutaneous soft tissue tumours in dogs: classification, differentiation, and histogenesis. Vet Dermatol 9:43–48, 1998
4. Kuntz CA, DernellWS, Powers BE, et al: Prognostic factors for surgical treatment of soft tissue sarcomas in dogs: 75 cases (1986–1996). J Am Vet Med Assoc 211:1147–1151, 1997
5. Dernell WS, Withrow SJ, Kuntz CA, et al: Principles of treatment for soft tissue sarcoma. Clin Tech Small Anim Pract 13:59–64, 1998
6. Connery NA: Surgical management of hemangiopericytoma involving the biceps femoris muscle in four dogs. J Small Anim Pract 43:497–500, 2002
7. Bostock DE: Prognosis after surgical excision of canine fibrous connective tissue sarcomas. Vet Pathol 17:581–588, 1980
8. Postorino NC, Berg RJ, Powers BE, et al: Prognostic variables for canine hemangiopericytoma: 50 cases (1979– 1984). J Am Anim Hosp Assoc 24:501–509, 1988
9. Graves GM, Bjorling DE, Mahaffey E: Canine hemangiopericytoma: 23 cases (1967–1984). J Am Vet Med Assoc 192:99–102, 1988
10. Fossum TW, Couto CG, DeHoff WD, et al: Treatment of hemangiopericytoma in a dog, using surgical excision, radiation, and a thoracic pedicle skin graft. J Am Vet Med Assoc 193:1440–1442, 1988
11. Forrest LJ, Chun R, Adams WM, et al: Postoperative radiotherapy for canine soft tissue sarcoma. J Vet Intern Med 14:578–582, 2000
12. McKnight JA, Mauldin GN, McEntee MC, et al: Radiation treatment for incompletely resected soft-tissue sarcomas in dogs. J Am Vet Med Assoc 217:205–210, 2000
13. Bacon NJ, Dernell WS, Ehrhart N, et al: Evaluation of primary re-excision after recent inadequate resection of soft
tissue sarcomas in dogs: 41 cases (1999–2004). J Am Vet Med Assoc 230:548–554, 2007
14. Dernell WS, Withrow SJ, Straw RC, et al: Intracavitary treatment of soft tissue sarcomas in dogs using cisplatin biodegradable polymer. Anticancer Res 17:4499–4504, 1997
15. Plavec T, Kessler M, Kandel B, et al: Palliative radiotherapy as treatment for nonresectable soft tissue sarcomas in the dog—a report of 15 cases. Vet Comp Oncol 4:98–103, 2006
16. Gillette SM, Dewhirst MW, Gillette EL, et al: Response of canine soft tissue sarcomas to radiation or radiation plus hyperthermia: a randomized phase II study. Int J Hyperthermia
8:309–320, 1992
17. Stefanello D, Morello E, Roccabianca P, et al. Marginal excision of low-grade spinale cell sarcoma of canine extremities: 35 dogs (1996-2006). Vet Surg. 2008 Jul;37(5):461-5.
18. Banks T, Straw R, Thomson M, et al: Soft tissue sarcomas in dogs: a study assessing surgical margin, tumor grade and clinical outcome. Aust Vet Pract 34:142–147, 2004
19. Selting KA, Power BA, Thompson LJ, et al: Outcome of dogs with high-grade soft tissue sarcomas treated with and without adjuvant doxorubicin chemotherapy: 39 cases. JAVMA 227(9):1442-1448, 2005

Mast Cell Tumours: How do we manage them in relation to margins?

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Traditional guidelines for the treatment of mast cell tumour have advocated 3cm lateral margins and resection of a deep fascial plane. The origin of this 3cm rule is unknown, and such prescriptive advice fails to fails to acknowledge that neoplasia represents a spectrum of disease. It would seem overly simplistic to suggest that a 3cm resection rule should apply equally to a 1cm biologically inactive MCT which has been present for 2 years, as it does to a 5cm mass that has been rapidly growing over the last few weeks. Although extensive surgery may undoubtedly achieve local cure in many cases, it may also result in significant and unnecessary morbidity which is all the more concerning if a similar result could be obtained with smaller resection margins. In addition, there may be some cases that do not receive potentially curative management due to anatomical, financial or other constraints if 'textbook' margins were strictly adhered to.

In 2005, Murphy and Brearley reported on the outcome of 340 mast cell tumours resected in general practice. In 70% of cases, incomplete or narrow surgical margins were performed yet no tumour recurrences were identified in these cases. This study supported the premise that wide surgical excision may not always be unnecessary to achieve successful clinical management in all cases, although is tempered by the consideration that about 40% of cases in this study were lost to follow-up.

Further challengers to the 3cm resection rule have emerged in recent years, with seminal work by Simpson et al (2004) who studied 23 MCT excised with 3cm margins. Comprehensive histological evaluation of the resected tissue at 1, 2 and 3cm about each 90° quadrant of resected specimen indicated that all Grade 1 tumours were excised with 1cm margins, and all Grade 2 tumours were completely removed with 2cm margins. These findings were independent of tumour size (range 0.35 – 5 cm). A subsequent prospective study achieved complete excision in 91% of cases with only 2cm skin/one fascial plane deep margins. (Fulcher 2006) Taking this a step further, a theory for "proportional margins" for tumour resection has also recently been proposed (Henderson 2008), whereby the width of the lateral skin margin is directly proportional to the tumour diameter – thus a 0.5cm diameter tumour is treated with 0.5cm skin margins, and a 2cm tumour is resected with 2cm margins. For tumours sized between 2-5cm, the results of previous work (Fulcher 2006, Simpson 2004) would suggest that 2cm margins were adequate. Nevertheless, it is important to state that it has not been studied whether a 2cm margin is sufficient for mast cell tumours greater than 5cm in size, or for Grade III tumours.

For many oncological conditions, it is becoming increasingly clear that the primary determinant of patient outcome is tumour *biology* rather than the extent of any surgery performed (Weitz and others 2003). Indeed, the failure of apparently adequate surgery to cure MCT was demonstrated by Weisse et al, (2002) who reported only an 89% local control rate despite complete excision being achieved in all cases. Other authors have reported similar rates of local tumour recurrence, despite apparently clean margins and adequate surgical dosing. The converse dichotomy is also true, however, with the recurrence of mast cell tumours following incomplete resection not being an inevitable event. Published works indicate that recurrence may occur in only 18-35% of cases

(Seguin 2006, Michels 2002). However, the importance of achieving adequate local control was demonstrated by Michels (2002) who showed that all tumour-related deaths in their study occurred following local relapse.

Mast cell tumours are a typically histologically circumscribed but unencapsulated tumour. Rather than being a monoclonal population of cells, each mass will be a heterogenous mixture comprising tumour cells, collagenous stroma and mesenchymal tissue, neovascular structures, inflammatory cells and extracellular matrix. A variable reactive zone will surround each tumour, which may extend only a few millimeters for small low grade tumours, to several centimeters for high grade degranulating tumours. Within the reactive zone will be normal inflammatory mast cells, which will be indistinguishable from their neoplastic cousins. Thus, the oncogenic potential of this reactive zone is unknown, and a 'clean' resection is considered if microscopically normal tissue is surveyed about the resected specimen.

The histological assessment of surgical margins as an indicator of complete tumour removal *in all planes* can be highly flawed, either as a consequence of processing methodology or the practical realities of a commercial laboratory service. For example, if a pathological specimen measuring 3x2x1cm was cut into 5 micron sections, it would take approximately 4000 histological sections to examine the entire tissue. In most commercial veterinary laboratories, reporting is performed on just 3-6 sections. Interpretation of the literature, where recurrence of a tumour has occurred in the face of an apparently 'clean' surgical resection must therefore be taken with caution.

Classically, histopathology is used to predict biological behaviour of a MCT, with the Patnaik grading scheme most commonly employed. In a blinded study, Northrup et al (2005) revealed poor correlation between pathologists when assigning a tumour grade, with agreement in only 6% of cases, with the same tumour being assessed as low-grade by one pathologist, intermediate grade by another, and high-grade by a third. Improved correlation was achieved following training but the authors concluded that a more objective grading system was required, and that other histological indicators of prognosis should be investigated.

The inability of histopathology to reliably predict grade, combined with evidence that 'big' surgery does not necessarily provide any certaincy of clinical cure, can confound the clinician who is faced with a mast cell tumour in their patients. Nevertheless, it remains a fundamental truism of oncological surgery that "the first surgical intervention" is optimal for the successful management of all tumours, and an inappropriate conservative resection may prove detrimental to the patient. Clearly it would be useful if further information was available to determine the biological tendency of an individual tumour. Such information would hopefully enable more precise individualization of therapy and prognosis for a patient, and assist the consideration of actual surgical dosing and the role of neoadjuvant or adjuvant therapies.

Ki67 is a proliferation marker that is tightly regulated to cell cycle events. It is a large protein, detectable by immunohistochemisty. Previous studies have indicated that expression of Ki67 by a tumour is significantly correlated with survival, and is independent of histological grade with a cut off at 1.8%. Thus, Ki67 may be used to determine those dogs that have long survival times compared to those with short survival times, and may therefore assist decision pathways for surgical management, and the role of adjuvant or neoadjuvant treatments.

Additional information on the likely behaviour of an individual tumour may be determined by the status of a cell surface, transmembrane protein kinase which functions as a major growth factor for mast cells. Between 9-33% of mast cell tumours have been shown to possess a mutation of the cKIT gene. Higher tumour grades are associated with more frequent cKit mutations (e.g. 30-50% of grade II/III MCTs will possess a cKIT mutation). Specific treatment with a TK inhibitor (e.g. masitinib) may improve prognosis for patients with an appropriate receptor mutation (Hahn et al, JVIM 2008, Webster et al, BMC Veterinary Research 2008). Development of PCR methods to detect those tumours expressing cKIT mutations may also assist tumour stratification in the pre-operative setting.

Reference list available on request: jpb@vetspecialists.co.uk

Poster Abstracts

Oncothermia basic research - Results of the in vitro experiments

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Objective: Heating by electric field is well known method in oncology. Its special technical realization (called oncothermia, OT) uses modulated RF-current conduction. Its selectivity is based on the explicit difference of dielectric properties of malignant and healthy tissue. Additional effects of OT are debated from its establishment. Our objective is studying OT in comparison to conventional hyperthermia (HT) on identical temperatures.

Material and Methods: Despite OT is a clinically successful method, its scientific modelling is rather complex. We applied two model systems to cover the complexity and make cross-control. The cell-lines were cultured on coverslip equipped with special electrode system. The changes of adherent connections were studied on monolayer of HepG2, A431, B16 malignant cell lines; while other cell membrane-associated effects (activation of the apoptotic signal transduction pathways, HSP-mediated stress-responses) were examined in suspension form. Experiments were accomplished on identical 42 °C reference temperature. The adherent connections were studied by immunohystochemical detection of E-cadherin and β-catenin using confocal laser microscope. The apoptosis study was based on V-Annexin detection by flow-cytometry.

Results: Definite difference was observed between OT and HT heated samples. OT significantly reestablished the adherent connections. Also special relocalization of β-catenin from the cell-membrane into nuclei was detected at OT after 24h of treatment; while such effect was not seen in HT. The observed distortion indicate for HT mainly necrotic-, while for OT mainly apoptotic mechanisms.

Conclusion: Results support the existence of thermally induced but not temperature dependent effects in the tumor cell destruction mechanism of OT.

Oncothermia basic research - Results of the in vitro experiments

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Objective: Electromagnetic hyperthermia (Oncothermia (OT)) is a less well known complementary method in cancer treatment. Our objective is studying the gene expression of two heat-shock proteins and 3 hypoxia-induced genes *in vitro* and *in vivo* after OT and "heat-only" conventional hyperthermia (HT).

Material and Methods: OT utilizes modulated 13.56 MHz RF-current conduction in capacitive coupled arrangement. Under normal conditions, heat shock proteins (HSPs) play important roles in normal protein folding, translocation of proteins across membranes, quality control in the ER and proteolytic turnover of many of the key regulators of cell growth and survival. They contribute to drug- and radio- resistance and in case of advanced cancers reflect a cytoprotective stress response to the hypoxic, acidic and nutrient-deprived environment. At molecular level, these proteins help cancer cells avoid apoptotic death. To reveal the effect of the treatments on these proteins at molecular level, our present preliminary studies focused on two members of this protein family: HSP70 and HSP90. Using real-time PCR we examine gene expression changes caused by OT and conventional hyperthermia (HT). To investigate how these treatments could influence hypoxia, we examined expression of three hypoxia-inducible genes: HIF1- α transcription factor, carbonic anhydrase CA9 and vascular endothelial growth factor (VEGF).

Results: Both HSPs showed increased gene expression, but a more significant difference (7-12 fold) was observed in case of HSP90, especially after OT at 42 °C; the two other treatments result similar gene expression increase.

Conclusion: Treatments have distinct effects on sample's gene expression, so we will examine other hypoxia-inducible genes to characterize the changing at molecular level.

Athos the lucky Boxer with low grade adenocarcinoma in the nasal cavity - a case study.

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Athos, an 11 year old male Boxer (having Hungarian owners working in England) presented following a 5 month history of a left nasal discharge with intermittent episodes of epistaxis, to the Animal Medical Centre Referral Services (Manchester, UK). The dog underwent a detailed physical examination, complete blood testing, histopathological examination and magnetic resonance imaging (MRI). The diagnosis was a low grade adenocarcinoma arising from the left nasal cavity. The malignant process was considered to be local, no metastases were found. Athos was generally in good condition, with no abnormalities on physical examination or blood tests. The referral veterinary advised local radiation therapy with palliative chemotherapy, and gave a prognosis of 8-25 months survival time.

For economical reasons the owners decided to treat their dog in Hungary. Cytoreductive surgery was performed at the Surgical Department, Veterinary Faculty (Budapest, Hungary) two months after the diagnosis. Post-surgical local irradiation (Cobalt-60, 6x4 Gray) was given at an Mo-We-Fri schedule at N.R.I.R.R. (Budapest, Hungary) starting 3 weeks after surgery. Electromagnetic hyperthermia (Oncothermia®) was done immediately before irradiations for 30 minutes to improve the efficacy of gamma irradiation. Histopathological examination of the excised tissue confirmed the initial diagnosis. Two months following the completition of radiation therapy a control MRI examination at the Institute of Diagnostic Imaging and Radiation Oncology of the Kaposvár University (Kaposvár, Hungary), showed no evidence of tumour tissue.

Athos, 7 months following the diagnosis seems to be symptom- and tumor-free except for an occasional left nasal discharge with no epistaxis.

Kazmer the unlucky tomcat with a fibrosarcoma in the cervical region – a case study.

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Kazmer, the 7 year old castrated male cat presented following a 2 month history of fast progressing neurological signs to the Department of Surgery, Veterinary Faculty (Budapest, Hungary). The cat underwent a detailed physical, neurological and orthopaedic examination, complete blood testing, myelography and CT examination. The suspected diagnoses was tumor involvement of the 5^{th} cervical vertebra. No metastases were found within the body.

Cytoreductive surgery was performed within one week after the diagnosis. Histopathological examination of the removed mass revealed bone originated low-differenciated fibrosarcoma. Two weeks after surgery the cat was referred into the National "F.J.C." Research Institute for Radiobiology and Radiohygiene for further diagnosis and treatments. Multiple scintigraphical examinations were carried-out by injecting ^{99m}Tc-MDP (Skeleton, Medi-Radiopharma), ^{99m}Tc-DMSA(5) (Penta DMSA, Medi-Radiopharma), and ^{99m}Tc-MIBI (Cardio-SPECT, Medi-Radiopharma). All the three radiopharmaceuticals showed significant focal uptake in the neck region indicating the presence of remnant tumor tissue locally but nowhere else in the body. In vivo ^{99m}Tc-MIBI wash-out examination and in vitro immunohistochemistry and real time PCR tests from the removed tumor proved the multidrug resistance of the case. Post-surgical local irradiation (Cobalt-60, 12x3 Gray) was given at an Mo-We-Fri schedule starting 3 weeks after surgery. There was no improvement in the neurological signs, and the cat started weight-loss so euthanasia was made on the owner's request.

Gross pathology, ex vivo nanoSPECT/CT examination of the excised neck region and further histopathological examinations were carried-out. However the case we present now proved to be totally non-treatable provided many experiences to the veterinary staff.

MHC class I antigen-processing machinery defects in feline primary mammary carcinoma

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Background: Tumor antigen processing and presentation by MHC class I antigen is a prerequisite for the recognition of tumor cells by cytotoxic T lymphocytes. Defects in MHC class I antigen processing machinery (APM) may provide tumor cells with an escape mechanism from immune surveillance. These phenomena have been convincingly documented in human tumors. On the other hand scant information is available about the expression of MHC class I antigens and APM components in animal tumors. Since this information contributes to our understanding of the role of immunological events in the pathogenesis and clinical course of malignant disease, in this study we have investigated the expression of immunoproteasome components and MHC class I antigens by feline mammary carcinoma cells.

Materials and Methods: Fifteen formalin-fixed, paraffin-embedded primary feline mammary carcinomas of different subtypes and autologous normal mammary epithelium were immunohistochemically stained with IFN-γ inducible proteasome subunits LMP2-, LMP7- and LMP10- and with MHC class I heavy chain-specific monoclonal antibodies.

Results: Normal mammary tissue showed strong cytoplasmic positivity for LMP2, LMP7 and LMP10, whereas MHC class I antigens were detected only on the cell surface. In contrast, MHC class I antigens, LMP2 and LMP7 were down-regulated in at least 80% of the neoplastic lesions tested. Furthermore, the level of LMP2 and LMP7 expression was significantly correlated with that of MHC class I antigen expression.

Conclusions: APM component abnormalities are present in feline mammary carcinoma lesions and may play a role in the down regulation of MHC class I antigens found in these tumors.

Redox status of feline tumors before and after photodynamic therapy.

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Photodynamic therapy (PDT) is a relatively new modality of cancer treatment in human and veterinary medicine. It uses a photosensitizing drug called photosensitizer, which in the presence of oxygen delivers reactive species (singlet oxygen and free radicals) upon irradiation of the tumor with visible light. PDT offers an additional method to treat patients with certain kinds of cancer.
Here we present data on the application of PDT on companion animals with malignant tumors.

We have applied 5-aminolevulinic acid (ALA)-based PDT on cats and dogs with different kinds of tumors, and investigated the effect of treatment on reduction-oxidation parameters of tissue samples taken from the tumors before and after PDT. Results of free radical concentrations measured using Electron Spin Resonance spectroscopy and of reduced glutathione in tumor tissues give a direct correlation with the outcome of PDT. No large tumors responded well to PDT as the efficacy of the treatment is limited by light penetration (about 1 cm at 600-700 nm). Smaller-sized tumors usually responded much better to a single treatment. We treated tumors in their very advanced and neglected stage, when no real cure could be expected.

Qualitative data obtained on the correlation between total free radical/GSH concentrations and the outcome of PDT suggests the possibility of a good prognostic value for evaluating the effectiveness of the treatment.

Radiotherapy successfully controlled a recurrent hemangioma in a juvenile Standardbred colt

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Introduction: Hemangiomas of the skin in young horses are rare and only two cases affecting the coronary band are documented in the literature. These vascular tumors may be present at birth or occur during juvenile development. Further, this neoplasia has the potential to recur when a wide surgical excision cannot be performed.

Material and methods: A 13-month-old standardbred colt was presented to the University of Veterinary Medicine Vienna with the second recurrence of a hemangioma at the level of the coronary band of the right hind limb. The third attempt to excise the tumor with wide margins failed and resulted in a non-healing skin and hoof defect. Hypofractionated radiotherapy (6 x 6 Gy) was administered to the recurring tumor in the wound-bed.

Results: The open wound slowly retracted after the end of radiotherapy and needed about 4 months for complete closure. The colt started race training 6 months post treatment and competed in his maiden race 14 months post radiotherapy. No recurrence occurred in the first 18 months of follow-up. A hypo-pigmented band in the hoof capsule and mild alopecia in the skin near the coronary band developed as a mild chronic side effect from radiation.

Conclusion: Hemangiomas in young horses seem to be radiosensitive. Radiation therapy can be considered in cases where surgical intervention is not curative.

Determination of tumor size and perfusion as in vivo biomarker in mouse tumor models

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Introduction. We examined the possibilities of monitoring lesion size and blood perfusion as biomarkers for anti-angiogenic treatment in mouse model of metastatic hepatic cancer and subcutaneous melanoma. As the NanoSPECT/CT® hybrid imaging device is capable of accurate absolute quantification of radioactivity in vivo, we decided to use quantification of radiolabelled serum albumin uptake as marker of perfusion.

Methods. Dedicated small animal SPECT/CT scans were made 3h post injections. Subcutaneous tumor models were represented by 3x5 mice with subcutaneous melanoma with two types of treatment, erythropoietin plus Avastin (E+A) or Avastin (A) alone. Animals were scanned at start and 1 week post start of therapy. Tumor perfusion was defined as absolute activity content in the tumor compared to one part of the (clearly identified) aorta.

Results. Tumor perfusion scans offered in vivo estimation of blood distribution revealing the peripherial perfusion and central hypoperfused areas. Treatment groups did not differ significantly in terms of tumor size or perfusion while a small difference was detected between the treated and the control groups' tumor perfusion (8.12%+-2.34%)

Conclusions. The dedicated small animal SPECT/CT is an excellent tool to perform in and ex vivo non invasive studies in small animal tumor research. The relative uptake calculation allowed accurate identification of in vivo biomarkers of a model for further tumor pharmaco-therapeutical screening.

Clinical pathological findings in a dog with unilateral renal carcinoma

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Background: A 7-year-old, male, German shepherd with poor body condition and 5-months history of intermittent hematuria was admitted for a complete clinical evaluation.

Methods: Complete blood cell count (CBC), biochemical and coagulation profiles, screening tests for infectious diseases were performed at the admission. Plain view thoracic, abdominal radiographs and abdominal ultrasonograph examination were made one week later. Cytological examination, nephrectomy and histopathological evaluation of the right kidney were performed. Continued monitoring was suggested for the following weeks.

Results: At the admission a non regenerative normocytic hypochromic anemia, moderate leukocytosis, marked hypoalbuminaemia and hematuria were seen. Coagulation test results were suggestive for a disseminated intravascular coagulation (DIC). Serial CBCs showed a persistent anaemia associated with a worsening extreme leukocytosis. The abdominal ultrasonography revealed an enlarged, irregularly shaped and cavitated right kidney. Cytological features were suggestive of a renal carcinoma which was histologically confirmed according to WHO International Histological Classification. Following nephrectomy the dog was clinically improved and laboratory results normalized.

Discussion: Primary renal carcinoma account approximately for 0.5 % of all cancers in dog. We report a case of a renal carcinoma associated with paraneoplastic extreme leukocytosis, and DIC without dermatofibrosis. Paraneoplastic extreme neutrophilic leukocytosis is an uncommon finding which can be caused by the overproduction of granulocyte-colony stimulating factor by neoplastic cells. DIC could be also considered paraneoplastic in origin such as reported in humans. Because of these facts clinicians should be alert to the occurrence of these uncommon paraneoplastic syndromes and should be familiar with their diagnosis and management.

Cell proliferation and apoptosis in Canine Cutaneous Histiocytoma regression.

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Background: Canine cutaneous histiocytoma (CCH) is a common, benign epitheliotropic tumour of Langerhans cells, with a rapid growth, frequently followed by spontaneous involution and complete regression.

Material and methods: In order to establish a possible role played by cell proliferation and apoptosis in CCH regression, a total of 93 canine cutaneous histiocytomas were analysed, categorized according to Cockerell and Slauson (1976) criteria, into four histological groups, representing different stages of tumour regression. The proliferation was evaluated by Ki-67 immunolabeling. The apoptosis was evaluated by TdT-mediated dUTP-biotin nick end-labeling (TUNEL) method. The differences in the proliferation and apoptosis were analyzed in the four histological groups.

Results: CCH showed a proliferation index of 23,56±7,91%, evaluated with Ki-67 antigen. The apoptotic index was 39,37%±5,87%, detected by the TUNEL method. The proliferative and apoptotic index did not correlate significantly. Although the percentage of Ki-67 and TUNEL positive cells was similar among the different histological groups, the apoptotic index was always superior to the proliferative index in the groups considered.

Conclusion: The present study shows a similar pattern of cell proliferation (Ki-67) and apoptosis in the different stages of CCH regression. Nevertheless, the apoptotic activity exceeds cellular proliferation reflecting that apoptosis could play a significant role in spontaneous regression of CCH.

Radiotherapy and chemotherapy for the treatment of a cutaneous MCT in a dog.

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An eight-year old, female Labrador retriever was referred regarding rapid onset unilateral facial swelling. A small (1x1 cm) cutaneous lesion had been present on the lip for at least 6 months without any other signs. There was no response to initial treatment with antibiotics and anti-inflammatory drugs for two weeks so the primary clinician referred the case.

A diagnosis of MCT was made using FNA of the original lesion. Metastasis to the cervical and the pre-scapular lymph nodes was confirmed by FNA and a CT-scan. No other metastases were detected on CT or ultrasound scan. The patient was immediately started on treatment with oral prednisone and misoprostol in preparation for radiotherapy. The dog received four fractions of 9Gy to the muzzle and neck (primary and drainage lymph nodes), at weekly intervals. Adjuvant chemotherapy was given using oral prednisone daily and intravenous vinblastine (VBL) administered once weekly. A partial (almost complete) remission was obtained and maintained for four months. The protocol was then switched to oral lomustine (CCNU) every three week as recurrence of the metastatic disease became apparent. A partial remission was again achieved.

The only adverse effects of treatment were typical skin and mucosal changes from the radiotherapy. Neither chemotherapy protocol caused a problem. The dog died from sudden onset of respiratory distress 177 days after starting specific treatment, having led a normal happy life throughout treatment.

Establishment and characterization of a novel canine B-cell line derived from a malignant non-Hodgkin's lymphoma.

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Introduction: Canine lymphoma is the most common spontaneously occurring hematopoietic neoplasia in dogs. The tumor incidence is with 13-33 cases in 100.000 dogs per year much higher than in humans. However, in veterinary medicine *in vitro* studies have been limited by the lack of validated, well characterized, and widely available canine lymphoma cell lines as a baseline for better investigation of this disease.

Material and methods: To further accelerate the use of spontaneous cancers in dogs as a model for cancer cell biology and new anti-cancer drug development, we established a B-cell lymphoma cell line (CLBL-1) from a dog with a multicentric B-cell lymphoma. This CLBL-1 cell line shows a doubling time of approximately 24 h during exponential growth under standard culture conditions and is successfully maintained in continuous culture for more than half a year. For phenotypical characterization, CLBL-1 cells were stained with monoclonal antibodies against canine leukocyte-specific antigens and analyzed by flow cytometry.

Results: CLBL-1 were positive for CD11a, CD79αcy, CD45, CD45RA, MHC II and negative for CD3, CD4, CD5, CD8, CD11d, CD14, CD21, CD34, CD56 and TCR-γδ. PCR analysis for T-cell receptor (TCR-γ) and immunoglobulin heavy chain (IgH) gene rearrangements yielded a monoclonal result (single band) for the IgH gene and a negative result for the TCR-γ gene.

Conclusion: This cell line, representing one of the rarely reported canine lymphoma cell lines, displays the phenotype of B cells but not T cells and will help to promote translational and comparative lymphoma research in dogs and humans.

Histological Prognosticators in Feline Cutaneous and Visceral Mast Cell Tumors

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Background. Mastocytomas (MCTs) are common tumors of the skin, intestinal tract, and spleen in cats. Histologically, feline MCTs are classified as mastocytic (well differentiated or poorly differentiated/pleomorphic) and histiocytic/atypical. The biological behavior ranges from benign to malignant, but prognostic factors are not well defined.

Methods. Cell differentiation, growth pattern, infiltrating eosinophils, lymphoid aggregates, erythrophagocytosis and collagen degeneration were evaluated retrospectively in 48 feline MCTs. Immunohistochemistry was also applied to assess KIT pattern (CD117), cell proliferation (Ki67/MIB1-index) and telomerase activity (hTERT). Follow-up information was obtained via telephone interviews.

Results. Histologically, there were 36 cutaneous MCTs (18 well differentiated, 10 pleomorphic and 8 atypical), 7 splenic (5 well differentiated and 2 atypical) and 5 intestinal (1 pleomorphic and 4 atypical). Immunohistochemistry revealed cytoplasmic expression of CD117, either focally clustered or diffuse, in 91% of cases; proliferative activity and inflammatory infiltrates were more marked in pleomorphic and atypical MCTs. h-TERT was expressed in 52% of samples, regardless of the histotype. Survival data were achieved in 26 cases. Six cats (23%) died because of tumor-related causes (5 pleomorphic and 1 atypical; 4 cutaneous and 2 intestinal). Ki67-index and inflammatory infiltrate were negatively associated to survival.

Conclusion. Our findings suggest that cell morphology (i.e. pleomorphic type), ***accompanying inflammatory*** infiltrate and Ki67 immunohistochemistry may be of help to decide which cases are to be considered more at risk and therefore require closer monitoring. Aberrant KIT localization and telomerase activity advise further exploration of these pathways as potential therapeutic targets.

CV247 a new adjuvant product for treating malignant tumour diseases of pet animals

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CV 247 preparation has been developed in Great Britain for the adjuvant therapy of dogs affected with malignant tumours by John Carter MRCVS and Dr Andor Sebesteny. CV 247 supports the antioxidant processes and thereby improves the quality of life of the affected animals and slows the progression of some tumours. Poster presents the results of clinical studies carried out in Great Britain and in Hungary.

For the studies dogs affected with malignant tumours (mastocytoma, malignant melanoma, mammary carcinoma and B cell lymphoma) were selected, whose owners did not want radio- or chemotherapy, or the tumour was already inoperable.

At the outset of the administration of CV 247 the type of tumour was determined by pathological examination. After the determination of the stage of the disease the quality of life of the animal was assessed and recorded on a subjective sliding grading scale at regular intervals both by the veterinarian in charge of the treatment and by the owner. During the treatment period the animals received a diet with reduced energy content. Their body weight was recorded regularly.

During the 6 month study involving above 70 animals so far, the majority of treated animals did not lose (or did not lose significantly) body weight and both the veterinarians and the owners observed and recorded an improvement in their quality of life. This was particularly noted in animals affected with mastocytomas, melanomas and mammary carcinomas while the treatment appeared to be less effective in cases of B cell lymphomas. The progression of the tumours was also notably slower in several cases.

Results so far suggest that CV 247 may represent a new alternative adjuvant therapy of malignant tumours in animals.

An unusual case of lung tumor- PNST in a golden retriever

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Our case was an eight-years old Golden retriever that suffered for three months from chronic coughing , dyspnoe, fever, weakness and tiredness. During a palpation of distal bones there was found a swelling, a soreness and a local heat. We made a laterolateral a ventrodorsal x-ray picture of the thorax to search for some malignancy. We also made a dorsopalmar and laterolateral view of a distal antebrachium.

There were found a palisade-shaped periostal reaction on distal extremities with a surrounding soft tissue swelling. In the thoracic cavity there was seen a circumscribed mass in the size of 16,5x11,5cm in the right medial and caudal lung lobe. It was responded a thoracotomy with potential lobectomy. The thoracotomy was done from the sixth intercostal space from the right side. With a progressive preparation and ligation there were cuted off the both involved lung lobes.

Histopathology proved a mesenchymal tumor from the group PNST, concretly a malignant schwannom. Then after owners mentioned, that there was histologically the same mesenchymal tumor before years in the region of left antebrachium that was excised. After surgery dyspnoe and coughing vanished, hematology and temperature came back to physiologic values. The soft tissue swelling of the distal extremities and their pain edged away. We continued with an adjuvant chemotherapy with nonencapsulted doxorubicin in the standard dose ($30mg/m^2$) in regime protocol- five times cure phases in three weeks interval.

Nowadays the patient completed the chemotherapy, is five months in the remission and is clinically healthy.

14-3-3 σ Expression in Canine Mammary Tumours

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14-3-3 σ, belongs to the 14-3-3 highly conserved acids proteins family, which are involved in the modulation of diverse signal transduction pathways. Loss of 14-3-3 σ expression has been observed in several types of human cancer, suggesting that it may play a role as a tumour suppressor gene. In contrast, other researches have reported a metastatic and invasion role of this protein in pancreatic cancer. Down-regulation of 14-3-3 σ protein due to hypermethylation of the CpG islands has been shown in various human cancers. The aim of the current study was to investigate the expression and the distribution pattern of 14-3-3 σ in canine mammary tumours and their metastasis.

Seventy mammary tumours (20 benign and 50 malignant) were analyzed using a polyclonal antibody against the N-terminus of the 14-3-3 σ isoform and the Biotin-Streptavidin system (B-SA). Immunohistochemical expression of 14-3-3 σ was expressed in the cytoplasm and occasionally in the nucleus of the myoepithelial cells in normal and dysplastic mammary gland and benign tumours. In malignant tumours, 14-3-3 σ was also observed in the cytoplasm of epithelial tumour cells, and both simple solid and anaplastic carcinomas were shown to present overexpression of the 14-3-3 σ. Furthermore, tumour cells within vessel lumen as well as metastasis to lymph nodes, liver and spleen showed high levels of 14-3-3 σ expression.

In conclusion, 14-3-3 σ appears to have an important role in canine mammary tumourigenesis and metastasis as is the case of some human cancers.

Immunohistochemical Analysis of COX-2 Activity in Several Tumours Types of the Dog and Cat.

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COX-2 is an inducible enzyme that is involved in the production of prostaglandins, which modulate pathologic events such as inflammation, wound healing, and neoplasia. Recent studies also demonstrate that nonsteroidal anti-inflammatory drugs (NSAIDs), which inhibit COX enzymes, can reduce the incidence of cancer in humans and experimental animal models and may be potential targets for therapeutic and preventive strategies. In order to be able to select patients as targets of therapy, as well as to know the real level of expression of COX-2 in a given tumour type, the standardization of the methods of study of COX-2 expression is mandatory.

In this work we present the results of the standardization of an immunohistochemical technique using a rabbit monoclonal antibody and the EnVision® detection system in routinely processed tissue samples of canine (Cn) and feline (Fl) tumours. Results were scored and the level of overexpression defined. Fl osteosarcomas, nasal and intestinal adenocarcinomas, squamous cell carcinomas (SCC), and Cn SCCs, melanomas, nasal, renal, prostatic, intestinal adenocarcinomas and transitional cell carcinomas of the urinary bladder were analyzed. In addition, clinical evolution (up to 9 months) of three selected cases, one feline and two canine SCC overexpressing COX-2 and treated with Firocoxib®, a selective COX-2 inhibitor, was assessed.

We conclude that our immunohistochemical method of detection of COX-2 activity in routinely processed tumour tissue samples from dogs and cats is a very sensitive and specific method which may be used to select patients amenable of adjuvant treatment with COX-2 inhibitors.

Molecular characterization and activation of AKT oncogene in feline mammary tumors

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Background: AKT is a member of protein kinase B family which results activated and amplified in several human tumors. Activation of AKT is associated to a poor prognosis and resistance to endocrine therapy and chemotherapy in human breast cancer. In breast cancer the activation of Akt is mediated mainly by EGFR1, HER2/neu and estrogen receptor alpha (ERα). It has been previously shown that feline HER2/neu is over-exspressed in 37% of feline mammary carcinomas (FMC) and it is correlated to poor prognosis. Aim of this study is to evaluate the activation of feline AKT (f-AKT) in feline mammary tumors and to characterize the sequence of feline f-AKT gene.

Methods: Activation of AKT protein was evaluated by immunohistochemistry on 80 FMC, 20 benign lesions, 3 metastases and 2 normal mammary gland. Western blot anti p-AKT and total AKT was performed on 5 FMC cell lines. Products of RT-PCR corresponding to f-AKT was automatically sequenced.

Results: Positivity to p-AKT was found in the 75% of tubulopapillary carcinoma. Western blot analysis revealed that all FMC cells lines showed activation of p-AKT. Partially sequence of f-AKT cDNA was determined and revealed a homology of 98% with canine and human AKT sequences.

Conclusion: These preliminary data showed that AKT is activated in a high percentage of FMC and that its activation is correlated to malignant behaviour of tumoral cells. These data showed that f-AKT plays, as in human, an important role in the pathogenesis of FMC and confirming it as a suitable natural model in comparative oncology.

SURGICAL ONCOLOGY – PAST, PRESENT AND FUTURE

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Biology is King; selection of cases is Queen, and the technical details of surgical procedures are the Princes and Princesses of the realm who frequently try to overthrow the powerful forces of the King or Queen, usually to no long-term avail, although with some temporary apparent victories. (Cady, 1997)

It is a sobering thought to realise that one has reached the advanced stage in a professional career where one can be considered fair game to be invited to give 'perspective' overviews of the development of a discipline! However, I take some comfort in the fact that organised veterinary oncology is a relatively recent development still really only in its fourth decade. Despite the fact that cancer in animals has been recognised for considerably longer than the condition in man, it was only in the 1970's that one can really identify the first significant steps being taken to recognise and treat the cancer as a separate disease entity. Groups in veterinary schools both in the US and Europe at this time began to mould the discipline although it must be said that this was sometimes against a background of considerable criticism from their colleagues who perceived this new discipline as an unnecessary means of prolonging patient suffering. We have considerable reason therefore to be grateful to pioneers such as Brodey, Gillette, Madewell and Theilen in the US and Owen and Misdorp in Europe without whose vision and thick skin veterinary oncology would never have been born.

Until the advent of an animal tumour staging system less than thirty years ago (Owen, 1980) a simplistic view of surgical cancer management prevailed. This was that cancer spread via the regional lymph node and it was felt therefore that bigger surgeries would inevitably result in greater chances of a cure. Without a comprehensive understanding of cancer biology that included staging, grading and individual behaviour, surgical cancer management in animals was usually undertaken in a cautious manner with little realistic prospect of predicting the outcome. The first clinical staging protocols, created under the auspices of the World Health Organisation were part of the long road that began our improved understanding of cancer behaviour and allowed us to adopt a more rational approach to the surgical ablation of solid tumour disease.

It is not easy to identify the precise landmarks that have allowed the subsequent development of veterinary surgical oncology however the various roles (biopsy, prophylaxis, definitive, cytoreduction and palliation) that surgery could play in the management of neoplastic disease were quickly recognised. These roles began to replace the old approach of a universal 'cancer procedure' in which several, indeed sometimes all, of these roles were often simultaneously combined. The concept that biopsy was generally a 'bad' thing and could promote dissemination and 'upgrading' of the disease was gradually replaced as the recognition that tumour behaviour could be reliably predicted preoperatively and improved techniques were eventually developed.

In parallel with the concept that oncology could be a clinically important subject, much information was being published from veterinary pathologists such as Brodey, Patnaik and Bostock in the 70's that allowed improved understanding of basic tumour behaviour concepts such as grade, and the impact of tumour location and growth characteristics. Stemming from this, came the realisation that different tumour types / grades would require different surgical margins to accommodate their varied biological behaviour and mode of growth for cure. The development of local, wide local and compartmental margins, reflected today in the logo of the Veterinary Society of Surgical Oncology, became a norm and the much-needed justification for more aggressive and comprehensive surgeries.

Advances in related clinical and paraclinical areas, although perhaps somewhat less momentous, have continued to have an important impact on the continued refinement and accuracy of our surgical planning and the extent of the surgeries that are now feasible.

- The development of more sophisticated diagnostic imaging techniques including ultrasound, sectional imaging (CT, MR, PET imaging etc) and isotope studies most of which are within easy reach of the veterinary oncologist today mean that we can image and stage cancer diseased with far more accuracy than ever before.
- Improvements not only in our methods for acquiring tissue samples but also in their interpretation in the pathology laboratory have meant ever more sophisticated means of assessing tumour classifications. The widespread and routine use of needle aspirates has heralded the advent of an entirely novel creature in the pathology lab, 'the cytologist' who could interpret them.
- The progressive realisation that many anatomical structures would be sacrificed with some impunity and still allow the patient to continue a good quality of life that owners would find acceptable allowed a steady stream of more radical surgeries - mandibulectomy, limb sparing, hemipelvectomy, maxillectomy, orbitectomy, sinusectomy, chest wall resection, partial glossectomy all seem relatively commonplace today. Some procedures with considerable morbidity on the other hand, for instance radical mastectomy in the bitch, were abandoned when it was realised that this seemingly logical aggressive surgery had little or no impact on prognosis.
- Increasingly more radical surgeries have demanded a greater ability to repair tissues and the progress in reconstructive surgery in the past two decades have added enormously to our ability to undertake ever more extensive surgeries. In particular, one can highlight improvements in cutaneous reconstruction and Pavletic's work to describe the vascular anatomy in small animals has meant that there are now few areas of the body where it is not possible to create a flap of some description. The increasingly availability and sophistication of prosthetic materials for reconstruction of both soft and bony tissues add to our armamentarium of reconstructive options.
- The progressive advances in anaesthesia, analgesia and critical care have also contributed to our ability to undertake ever the more radical and extensive surgeries.

Today, there is an increasingly realisation that successful cancer management requires not just the work of one clinician but the combined efforts of a multi-modality oncology team and the cancer surgeon is just one member of that team. More and more veterinary hospitals are therefore following the example of our medical colleagues and putting together teams capable of planning and executing all aspects of cancer therapy.

Our advances in cancer management can only be properly appraised through randomised prospective clinical trials and such trials are much more efficiently and rapidly undertaken with input from groups of cancer centres. The advent of organisations such as The Veterinary Cancer Society has provided an important forum for such clinical trials.

Similarly, the training of future surgeons specialising in oncology is important for the continued progress of veterinary oncology and teams such Colorado State have provided leadership in the development of such training centres

Given the important role that surgery has played in oncologic surgery to date, it is perhaps surprising that it is only comparatively recently that a society dedicated specifically to this area has be founded. Perhaps surgeons are the last of the oncology team to feel the need to cooperate! Nevertheless, the VSSO now promotes training for oncologic surgeons, multi-centre prospective trials and cooperation between centres to pool resources and experiences. If we look ahead to the future it is difficult to believe that oncologic surgeons will retain their role as the primary veterinary oncologic clinician even in the management of solid cancers for much longer. Steve Withrow's confident statement even as recently as 1999 that 'The mainstay of local disease control for animals with cancer has been **and** will continue to be surgery, with radiation therapy gaining ground but still a distant second' begins to look less and less certain. Certainly, this will be the case for the foreseeable future but given the advances in gene therapy and the more sophisticated targeting of therapies, it seems that surgeons may have to adapt to a role that is increasingly multimodality-based and enabling based.

TUMOURS OF THE CHEST WALL

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The thoracic wall is a strong and compliant structure which consists of thirteen paired ribs, the intercostal muscles between them and eight sternebrae. The first nine ribs articulate with the sternum. The costal cartilages of the next three ribs lie close to the sternum, but the thirteenth rib is a short, floating rib.

Tumour Disease
Neoplastic lesions of the chest wall are very uncommon in the dog and even more so in the cat. Almost without exception they are sarcomas, the most prevalent types being:

- chondrosarcomas (CSA)
- fibrosarcomas (FSA)
- osteosarcomas (OSA)
- haemangiosarcomas (HSA)
- undifferentiated (UnSA)

(Matthiesen et al, 1992; Pirkey-Erhart et al, 1995; Baines, Lewis & White, 2002) and all therefore, potentially carry a guarded prognosis. They most commonly involve the ribs and often arise rapidly. The mean age of affected dogs is young (6 - 7 yrs) with no sex or breed predilection although they tend to be mainly medium to large breeds. Any rib can be affected with 4, 5 & 6 are most common and 1 & 2 least common.

Clinical signs
Tumours involving the chest wall present as obvious distortions of the chest contour. The mass itself may be painful and occasionally the patient is presented with a history of local trauma. The most important presenting sign is an enlarging mass but other signs include:

- lameness,
- dyspnoea,
- cough,
- pleural effusion and
- exercise intolerance.

Staging and Diagnostic Work Up
Diagnosis using incisional biopsy is important for differentiation from non-neoplastic lesions. Although the histological diagnosis will not dictate treatment mode since surgery is the only option, it will however have a considerable impact on prognosis (best: CSA> FSA> OSA worst) and this may determine if treatment is to be undertaken.
Radiographic examination using standard lateral and dorso-ventral views supplemented by skyline views of the lesion may demonstrate:

- which rib is primarily involved
- destruction of the rib or
- 'splaying' of adjacent ribs.

The lung fields should be scrutinised carefully for evidence of secondary disease since 10% of patients have secondary disease at the time of initial diagnosis (Baines, Lewis & White 2002). Sectional scanning (MRI, CT) is invaluable in assessing local involvement of diaphragm, abdominal viscera. Metastasis checks should include the following sites:

- Lungs
- Bronchial nodes
- Other ribs

- Other skeletal sites

Where necessary, isotope studies may be needed to identify metastatic disease.

Surgery

The consistent treatment modality for rib tumours is surgery (chest wall resection). In cases of localised tumours without evidence of metastasis surgical excision with chest wall resection may be appropriate. The mass should be removed intact with at least one additional rib and undisturbed intercostal tissue on either side of it. This may dictate resections of up to 6 ribs and create a significant deficit.

Reconstruction

Several goals have been identified as important for successful chest wall reconstruction;
Successful reconstruction of the chest wall was originally thought to necessitate all of the following:

1. Airtight seal
2. Closure of dead space
3. Stable chest wall
4. Protection of thoracic organs
5. Acceptable cosmetic result

Today only 1 & 2 are considered important and a number of techniques are available for reconstruction of the deficit to achieve these goals:

- **Primary Closure:** In the unusual event that only one or two ribs are removed it may prove practical to simply co-apt the wound although this normally creates some tension at the site. If the ribs cranial and caudal to the defect can be apposed without undue tension, then simple primary closure may be used. Encircling sutures are passed around the ribs cranial and caudal to the defect and tied to maintain approximation of the ribs. A rib approximator may be used to maintain the ribs in apposition during closure. The surrounding muscles (intercostal, latissimus dorsi, pectoral, external abdominal oblique) may be mobilised and sutured over the defect to provide additional protection. Primary closure is difficult to achieve if more than one rib has been removed.
- **Diaphragmatic advancement:** This is a comparatively simple technique for reconstruction of the chest wall and is suitable for tumours involving ribs 9 - 13. Following resection of the mass the diaphragm on the ipsilateral side of the costal arch is detached and drawn cranially in front of the thoracic deficit. The diaphragm is then sutured to the first intact rib in front of the deficit effectively closing the thoracic cavity. The abdominal deficit is closed by mobilisation of muscle and soft tissue from the abdominal wall.
- **Soft Tissue Augmentation:** deficits can be closed using autogenous soft tissue structures, including:
    - Latissimus dorsi muscle: where not involved in the tumour process, mobilisation of this muscle has proved to be the most popular means of closing the chest wall defect. The distal and dorsal attachment of the muscle is carefully released ensuring that the vascular supply remains intact. The pedicle in then reflected over the defect and anchored in place.
    - Omentum: although less commonly used today, the omentum can be lengthened and tunnelled via a paracostal thoracotomy to reach the defect and used to seal the chest wall (Bright and others, 1982).
    - External abdominal oblique muscle: this muscle is usually utilised where diaphragmatic advancement has been employed to permit closure of the resulting abdominal wall defect.
- **Prosthetic reconstruction:** The use of plastic spinal plates as prosthetic ribs was originally described (Ellison and others, 1981) but such extensive re-stabilisation is now considered unnecessary today. A variety of permanent or absorbable mesh materials including polypropylene, carbon fibre and polyglactin can be used to fill the deficit. The mesh material is sutured to either the pleural surface or outer surface of the thoracic

deficit and then covered with the latissimus dorsi muscle and subcutaneous tissues to close the dead space over the reconstructed area. Prosthetic materials are used less and less frequently since they represent a more complex technique than muscle flaps.

Prognosis

The prognosis for dogs with chest wall sarcomas is profoundly influenced by histological type:

OSA: The biological behaviour of chest wall osteosarcoma is similar to that of the appendicular form, with a 6 mth survival rate of 14 - 20% (Feeney et al, 1982; Matthiesen et al, 1992; Pirkey-Erhart et al, 1995; Baines, Lewis & White, 2002). Adjunctive chemotherapy with cisplatin increases the disease-free interval and survival up to approximately 9 months.

FSA: the prognosis for dogs with fibrosarcoma is somewhat more favourable with a 6 mth survival rate of 50%.

CSA: chondrosarcoma is associated with a much more favourable prognosis, with median survival times of up to 3 years.

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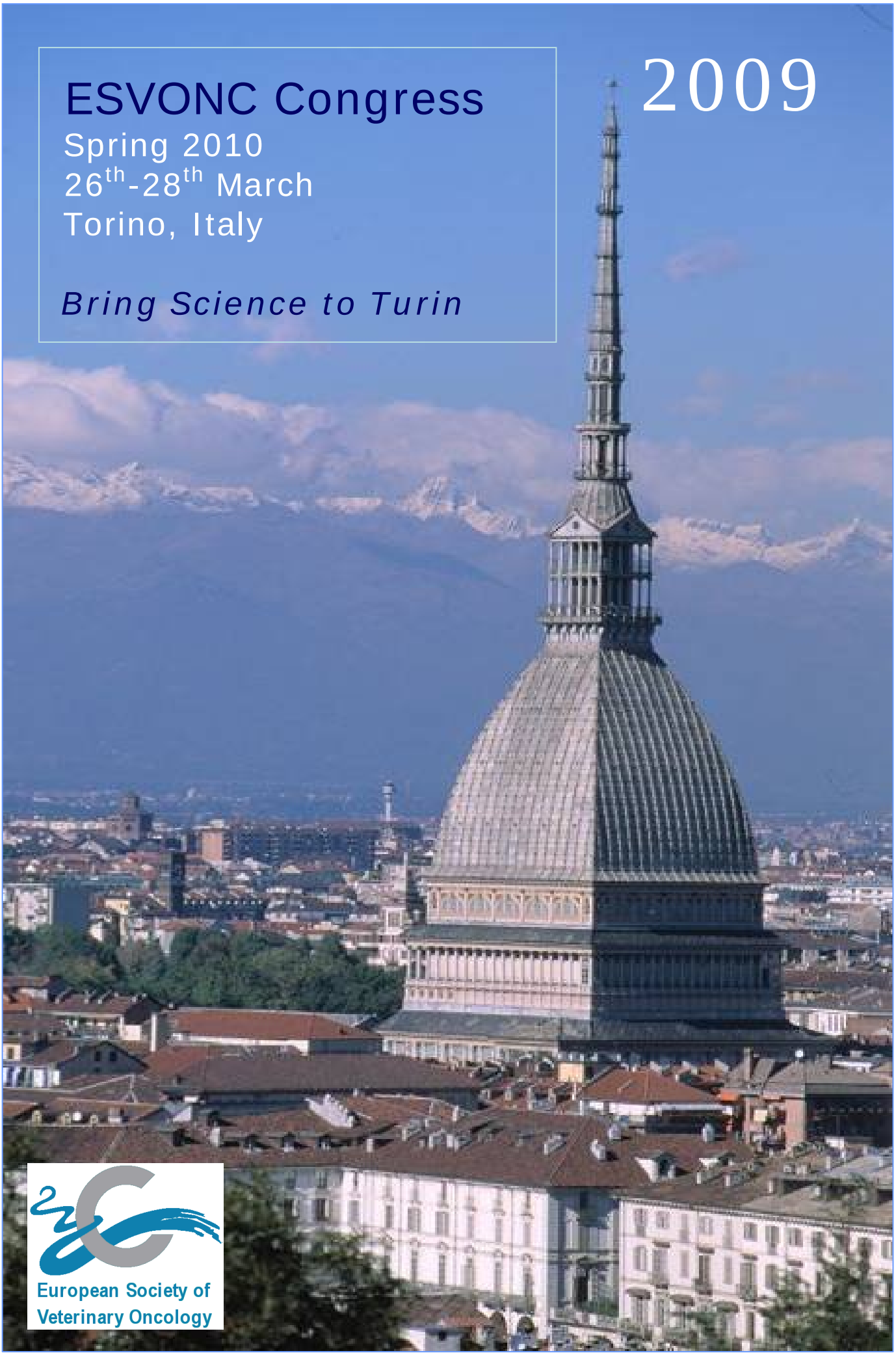

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