Targeting multiple oncogenes simultaneously improves response to therapy and circumvents acquired resistance to single target tyrosine kinase inhibitors

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European Society of Veterinary Oncology

Proceedings

22nd – 24th May 2014

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Vienna, Austria
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The organising committee wish to thank all those persons who helped review the submitted abstracts, judged the Residents’ competitions, moved chairs, carried things and generally made the smooth running of the conference possible.

And finally many thanks to the staff of the congress venue.

Many thanks also to our printers

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# Thursday 22nd May

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Dear colleagues and friends,

Welcome to the annual ESVONC congress being held for the first time in Vienna! We will, as has been the case for the past many years, have a great program with state of the art information from different fields of veterinary and comparative oncology. The main focus of the congress is, as always, to put together the recent knowledge on basic sciences and clinical oncology. As you already know, based on the remarks of last year’s questionnaire, we have decided to pursue with two items, that were really appreciated in the last congress in Lisbon: first, the themed sessions with a focus on lower urinary tract tumors on Thursday and melanoma on Saturday; second, there will be invited clinical abstracts, selected by our scientific committee.

The local committee, headed by Miriam Kleiter, has the tremendous responsibility to organize, along with our ESVONC team, this academic program that is top of the world as well as a great social program in this wonderful town! Don’t miss it! You know that ESVONC Annual congress is a place not only for scientific meeting but also for social gatherings and sharings: the venue will be at the splendid Palais Niederösterreich, the Welcome Reception will be in the Spanish Riding School! On Friday evening, we will organize our annual gala dinner in the Palais Ferstel.

And I can promise you one thing: all these nice people working for this congress and these well organized things will contribute to make for everyone of you and us, some « Happy congress Days »!

ESVONC President Frederique Ponce and the ESVONC team
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**Canine Urinary Bladder Tumors**

Urinary bladder cancer accounts for approximately 2% of all reported malignancies in the dog.\(^1\) With >100 million pet dogs worldwide, even uncommon forms of cancer affect tens of thousands of dogs each year. Invasive transitional cell carcinoma (TCC), also referred to as urothelial carcinoma, is the most common form of canine urinary bladder cancer.\(^1,2\) Most TCCs are intermediate- to high-grade papillary infiltrative tumors.\(^1,2\) Low grade superficial TCC, which is common in humans, is rare in dogs. Some of the most exciting work in canine TCC, however, is comparative oncology research that is expected to positively impact the outlook for dogs and humans with invasive bladder cancer. Other bladder tumors in dogs include squamous cell carcinoma, adenocarcinoma, undifferentiated carcinoma, rhabdomyosarcoma, lymphoma, hemangiosarcoma, fibroma, and other mesenchymal tumors.\(^2\) This discussion will focus on invasive, high grade TCC.

TCC is most often located in the trigone region of the bladder. Papillary lesions and a thickened bladder wall are common and can lead to partial or complete urinary tract obstruction. TCC of the bladder has been reported to also involve the urethra in 56% of dogs and the prostate in 29% of male dogs.\(^3\) Nodal and/or distant metastases are present in ~15% of dogs at diagnosis and 50-60% of dogs at death.\(^3\) Following World Health Organization (WHO) criteria for staging canine bladder tumors\(^4\), approximately 75% of dogs have T2 tumors, and 25% of dogs have T3 tumors.\(^3\)

The etiology of canine TCC is multifactorial. Risk factors include exposure to older-generation flea control products and lawn chemicals, obesity, possibly cyclophosphamide exposure, female gender, and strong breed-associated risk.\(^1\) In a recently updated analysis of cases in the Veterinary Medical Data Base (university based veterinary hospitals in the United States and Canada), breeds of dogs with significantly higher TCC risk compared to mixed breed dogs included: Scottish Terrier (OR 21.1, 95% CI 16.3-27.5), Eskimo Dog (OR 6.6, 95% CI 3.3-13.0), Shetland Sheepdog (OR 6.0, 95% CI 4.8-7.7), West Highland White Terrier (OR 5.8, 95% CI 4.2-8.1), Keeshond (OR 4.3, 95% CI 2.2-8.1), Samoyed (OR 3.43, 95% CI 1.8-6.5), and Beagle (OR 3.1, 95% CI 2.3-4.1) (unpublished data, Moore and Knapp, 2014). The female:male ratio of dogs with TCC has been reported to range from 1.71:1 to 1.95:1.\(^1\) TCC risk is higher in neutered dogs than in intact dogs of both sexes, although the reason for this has not been determined.\(^1,5\) A positive finding from a case control study in Scottish Terriers was that dogs that ate vegetables at least three times a week, along with their normal diet had a reduced risk of TCC (OR, 0.30, 95% CI 0.01-0.97, p <0.001).\(^6\) The specific type of vegetable with the most benefit could not be determined, but carrots, given as treats, were the most frequently fed vegetable in the study.

The most common clinical signs in dogs with TCC are hematuria, stranguria, and frequent urination.\(^1\) These same clinical signs, however, are more often associated with urinary tract infection, and can
also occur with urolithiasis and other bladder conditions. Many conditions can also mimic TCC in regards to abnormal epithelial cells in urine, and mass lesions within the urinary tract. Differential diagnoses include other neoplasia, chronic cystitis, polypoid cystitis, granulomatous cystitis/urethritis, calculi, and others. It is important to distinguish non-TCC conditions from TCC because the treatment and prognosis differ considerably and depend on the condition present. A definitive diagnosis of TCC is made through histopathologic examination. In poorly differentiated carcinomas, immunohistochemistry for uroplakin III (UPIII) can be helpful in distinguishing TCC from other carcinomas. Methods for obtaining tissue for histopathologic diagnosis include cystotomy, cystoscopy, and traumatic catheterization. These biopsy techniques are discussed under the surgery section and below under interventional urology. When obtaining a biopsy of suspected TCC, care should be taken to avoid seeding the tumor. In a recent report, 24 of 544 dogs with TCC evaluated at the Purdue University Veterinary Teaching Hospital (PUVTH), had spread of TCC to the abdominal wall. TCC in the abdominal wall developed significantly more often in dogs that had undergone cystotomy (18/177, 10.2%) than in those that had not (6/367, 1.6%). Once detected in the abdominal wall, the TCC grew aggressively and did not respond to medical treatments. The median survival after detection of the abdominal wall TCC in the 24 dogs was 57 days (range 0-324 days). The report also demonstrated that TCC can, albeit uncommonly, spread to the abdominal wall via lymphatics. With the poor outlook for dogs with abdominal wall TCC, every effort should be made to avoid seeding the tumor. Percutaneous biopsy methods and cystocentesis should be avoided if TCC is suspected.

Common clinical signs in dogs with TCC include hematuria, dysuria, pollakiuria, and, less commonly, lameness caused by bone metastasis or hypertrophic osteopathy. Urinary tract signs may be present for weeks to months and may resolve temporarily with antibiotic therapy. In dogs with TCC, a physical examination with rectal examination, may (or may not) reveal thickened urethra, enlargement of iliac lymph nodes, and sometimes a mass in the bladder or a distended bladder.

In dogs with confirmed or suspected TCC, evaluation should include an assessment of overall health (CBC, serum biochemistry profile, urinalysis, ± urine culture) and staging of the cancer (thoracic radiography, abdominal ultrasonography, and urinary tract imaging). To avoid the risk of seeding TCC through cystocentesis, urine may be collected by free catch or catheterization. If a catheter is to be passed, care must be taken to avoid penetrating the diseased bladder or urethral wall. Common sites of metastases detected with thoracic radiography and abdominal ultrasonography include lymph nodes, liver, and lungs, although metastases can occur in other areas as well including skin and bone.

Urinary tract imaging is used to assess the TCC location for potential surgical intervention and to map and measure TCC masses in order to subsequently determine response to medical therapy. Mapping TCC in the bladder, proximal urethra, and prostate can be accomplished by cystosonography, cystography, or computed tomography (CT). Regardless of the imaging technique used, to accurately track response to therapy it is important to follow a consistent protocol from visit to visit for bladder distension, patient positioning, equipment, and operator.

The treatment of TCC can include surgery, medical therapy, and radiation therapy. Although the trigonal location of TCC in dogs usually precludes surgical excision, surgery may be indicated for one or more of the following reasons: (1) to obtain tissue for diagnosis, (2) to attempt to remove the TCC within the bladder if lesions are away from the trigone, and (3) to maintain or restore urine flow. These are discussed more in the surgery section notes by Dr. Freeman. In the uncommon cases in which all gross TCC can be surgically removed, it appears helpful to place the dogs on a cyclooxygenase (COX) inhibitor to reduce the risk of recurrence from residual microscopic disease or from new primary tumors that form due to the field effect. In a small series of 9 dogs in which all gross TCC was removed, and then the dogs were treated with deracoxib (3 mg/kg daily), the median survival was 749 days (range, 231 to 2,581 days). Early reports of radiation therapy to treat TCC were not encouraging due to side effects, but new technology and growing expertise are improving
the application of radiation therapy to dogs with TCC. This is discussed more in the radiation therapy section by Dr. Larue.

Systemic medical therapy is the mainstay of TCC treatment in dogs and usually consists of chemotherapy, COX inhibitors (nonselective COX inhibitors and COX-2 inhibitors), and combinations of these. Although medical therapy is not usually curative, several different drugs can lead to remission or stable disease of TCC, and most therapies are well tolerated. Resistance to one drug does not necessarily imply resistance to other drugs. Some of the best results are seen in dogs that sequentially receive multiple different treatment protocols over the course of their disease. The approach used at the PUVTH is to obtain baseline measurements of the TCC masses, to initiate a starting treatment, to monitor the response to that treatment at 4-8 week intervals, and to continue that treatment as long as the TCC is controlled, side effects are acceptable, and quality of life is good. If cancer progression or unacceptably toxicity occurs, then a different treatment is instituted. Subsequent treatment changes are based on tumor response and treatment tolerability. By following this approach, TCC growth can be controlled in approximately 75% of dogs, the quality of life is usually very good, and median survival times can extend beyond a year. Although it could be tempting to simultaneously combine multiple chemotherapy agents in dogs with TCC, the benefit of this has not been determined, and the potential development of resistance to multiple drugs at the same time could limit subsequent therapy options.

Medical treatments that have been used commonly in dogs with TCC include: (1) single-agent COX inhibitor, (2) mitoxantrone combined with a COX inhibitor, and more recently (3) vinblastine with or without a COX inhibitor. The COX inhibitor that has been used most frequently in dogs with TCC is the nonselective COX inhibitor, piroxicam. Updated results of piroxicam treatment (0.3 mg/kg once daily) indicate a 20% remission rate with most being partial remissions, and 60% stable disease (lack of remission or progression) rate. Median survival times with single agent piroxicam are typically in the 6-7 month range with a minority of dogs living years after diagnosis. Quality of life is usually maintained or improved with piroxicam, although it is important for dog owners to observe the dog for potential side effects. The most common side effect which has been observed in approximately 15% of treated dogs, is GI irritation. If vomiting, melena, and anorexia occur, piroxicam should be withdrawn and supportive care provided as needed until the toxicity resolves. In these cases, it may be safest to switch to a COX-2 inhibitor if further COX inhibitor treatment is indicated. COX-2 inhibitors also have activity against TCC with reported activity of deracoxib, firocoxib, and others in dogs. The remission rate appears similar between COX-2 inhibitors and piroxicam, although complete remissions have been reported with piroxicam, but not with the COX-2 inhibitors to date.

Two of the most commonly used chemotherapy protocols in dogs with TCC are mitoxantrone combined with piroxicam, and vinblastine (alone or in combination with piroxicam). In a study of 55 dogs with TCC treated with mitoxantrone and piroxicam, 35% of dogs had remission with minimal toxicity, and the median survival was 291 days. In a study of 28 dogs treated with vinblastine, 36% of dogs had partial remission, and 50% had stable disease. Most dogs had failed other therapies when vinblastine was initiated, and the median survival from the start of vinblastine until death was 147 days. Data analyses are pending of a followup study in which dogs with TCC were randomized to receive vinblastine followed by piroxicam vs vinblastine given concurrently with piroxicam.

An alternative approach for the conservative management of TCC is metronomic chemotherapy with leukeran (4 mg/m² per day orally). Of 31 dogs with TCC, of which 29 had failed prior therapy, tumor control (1 partial remission, 20 stable disease) was accomplished in 70% of dogs. The median survival time from the start of chlorambucil to death was 221 days (range 7-747 days). Toxicoses were minimal with only one dog discontinuing therapy due to toxicity.
There are multiple other drugs that have antitumor activity against canine TCC. The platinum agents, cisplatin and carboplatin, appear more active (remission rates 40-70% when combined with COX inhibitors) than mitoxantrone and vinblastine. The reason the platinum agents are not used more often or used as front line treatment is because of undesirable side effects including bone marrow suppression, prolonged anorexia, other GI toxicity, and renal toxicity (cisplatin). Similarly, intravesical mitomycin C in dogs with TCC has resulted in remission and good tumor control, but in some dogs the drug is systemically absorbed. This unpredictable absorption results in severe systemic toxicity including bone marrow and GI toxicity. Other systemic therapies including doxorubicin and gemcitabine in combination with COX inhibitors have also been reported to have activity in dogs with TCC.

In addition to treating the cancer, it is important to monitor dogs with TCC for secondary urinary tract infections and to treat those appropriately based on culture and sensitivity. The emergence of highly resistant bacteria is posing an important and life threatening outcome in dogs with TCC.

**Feline Urinary Bladder Tumors**
Bladder cancer is rarely reported in cats. A report of 27 bladder tumors in cats included 15 carcinomas, 5 benign mesenchymal tumors, 5 malignant mesenchymal tumors, and 2 lymphomas. Most cats were elderly, and included 20 male and 7 female cats. A series of 20 cats with TCC included 13 neutered male and 7 spayed female cats (median age 15.2 years). A series of 15 cats with TCC examined at the PUVTH in recent years included 6 neutered male and 9 spayed female cats (median age 13 years, range 4-18 years). Clinical signs in cats with TCC are similar to those in dogs (hematuria, stranguria, pollakiuria). Concurrent urinary tract infection is common, being reported in 75% of cats in the published study and 67% of cats at the PUVTH. Regional and distant metastasis of feline TCC is clearly possible, but the metastatic rate has not been defined. The optimal treatment for bladder tumors in cats remains to be defined.

**Canine Prostate Cancer**
Prostate cancer is typically a disease of older dogs and can affect neutered and intact dogs. In broad terms, prostate cancer in dogs usually occurs in one of 3 forms: (1) TCC arising from prostatic duct epithelium, (2) adenocarcinoma that is clinically apparent and problematic, and (3) prostatic intraepithelial neoplasia (PIN) which is usually subclinical. TCC in the prostate is typically treated similarly to TCC in the bladder and urethra, although the response, as compared to TCC in the bladder, is not yet well defined. High grade PIN is an accepted precursor to prostate carcinoma in men, however, many men with PIN (especially if not high grade) do not progress to clinically relevant cancer. Step sectioning of the prostates from dogs with no evidence of prostatic disease who were euthanized for other reasons, revealed PIN, including some with high grade PIN. It is not known if these lesions would have progressed had the dogs not died of other causes. The prognosis for dogs that present with clinically problematic prostatic adenocarcinoma is poor. Canine prostatic adenocarcinoma is associated with a high metastatic rate including spread to bones, and poor response to traditional chemotherapy. Palliative care could include COX inhibitors and metronomic chemotherapy. Prostate cancer is very rarely detected in cats, and the optimal treatment has not been defined.

**Interventional Urology Applications for Lower Urinary Tract Tumors**
Interventional urology refers to image-guided, non-surgical procedures to correct or to provide accommodation for problematic urologic conditions. In regards to lower urinary tract tumors, this most often includes cystoscopic inspection and biopsy, and the non-surgical placement of urethral stents. Laser tumor resection techniques utilized in humans, have been described in dogs, but do involve potentially serious risks as discussed below. An interventional urologic approach to the placement of ureteral stents (i.e. insertion of ureteral stents via cystoscopy) could be considered, although technical challenges in small dogs and poor to absent visualization of the ureteral orifice due to the trigonal tumor, make this approach very challenging, if not impossible in some cases.
Cystoscopy provides the opportunity to visually inspect the urethra and bladder, and to obtain biopsies in a noninvasive method. The location of the tumor, distance from the trigone (which could allow subsequent surgery), and the extent of impingement of the tumor on ureteral orifices and urethra can be assessed. Small diameter flexible scopes are used in male dogs, and rigid scopes are generally used in female dogs. With the small size of cystoscopic biopsies, diligence in collecting sufficient tissue for diagnosis is crucial. Placing tissue samples in a histology cassette prior to processing helps prevent loss of small samples. In a report of 92 dogs, diagnostic samples were obtained by cystoscopy in 96% of female dogs and 65% of male dogs that ultimately had histopathologically diagnosed TCC. The more recent use of a wire basket designed to capture stones during cystoscopy allows collection of larger tissue samples and has increased the yield of diagnostic biopsy samples, especially in male dogs. Traumatic catheterization to collect tissues for diagnosis can also be performed, although samples collected by this method are usually small and the diagnostic quality varies considerably from case to case. Percutaneous biopsy methods can lead to tumor seeding and are best avoided.

Urethral obstruction from TCC will rapidly become fatal if not relieved. Procedures to relieve or bypass urethral obstruction include the placement of a cystostomy tube, or the placement of a urethral stent. The placement of cystostomy tubes is discussed in the notes on surgery. The advantages of urethral stents over cystostomy tubes are stents can be placed non-surgically with fluoroscopic guidance, and there are not any external components of stents for the dog to chew out. Currently, the most commonly used urethral stents are self-expanding nitinol mesh stents. These come in covered and uncovered designs. For the covered stents, the mesh in the middle of the stent is covered in order to theoretically reduce the growth of tumor through the stent mesh once it is placed. The ends of the covered stents are uncovered in order to engage the tissues to hold the stent in place. Problems with migration of covered stents, however, has led to increased use of uncovered stents. The uncovered stents appear more likely to stay in place, and tumor growth through the stents has only rarely been observed.

Briefly, for the placement of a urethral stent, the dog is placed under general anesthesia. A measuring catheter is placed in the colon and positioned parallel to the area of suspected urethral obstruction. A flexible tipped urologic hydrophilic guide wire is inserted up the urethra into the bladder. An appropriately sized vascular sheath (7 to 10 Fr) is placed over the guide wire. Iodinated contrast mixed 50/50 with sterile saline is infused into the bladder to achieve moderate bladder distention. A retrograde positive contrast urethrogram is then performed with fluoroscopy to identify the area of urethral obstruction. In female dogs, the vascular access sheath is withdrawn through the urethra during retrograde infusion of iodinated contrast agent to attempt to obtain maximal urethra distension and determine length and width measurements of the urethra. The colonic measuring catheter, which has 10 radiopaque bands at 1-cm intervals, is visualized on the urethrogram next to the urethra in order to determine the length of obstruction caused by the tumor mass, the length of the urethra measured from trigone to papilla in female dogs, and the widest diameter of urethra adjacent to the obstruction.

The width of the stent is selected to be at least 10% larger than the widest diameter of the urethra adjacent to the obstruction. When possible, the length of the stent is selected to allow 0.5 to 1 cm of the stent to be in contact with normal tissue proximal and distal to the obstruction. For female dogs with the entire urethra involved with neoplasia, the length of the stent is chosen with the goal to stent approximately 67% of the urethra, but to stent no more than 75% of the length of the urethra. Once the appropriately sized stent has been selected, the stent is placed over the guidewire, positioned across the obstruction, and deployed with fluoroscopic guidance. After stenting, the bladder is manually expressed to ensure urethral patency, and the dog observed several hours in the hospital.
The outcome of dogs with TCC with urethral stents has been reported in 2 recent publications. The first included 19 dogs at the PUVTH. \(^{24}\) Eleven dogs had already received medical therapy for their TCC at the time of obstruction. Urethral obstruction was successfully relieved in 17 of 19 dogs. In one of the dogs, the urethral obstruction was so severe that a guidewire (nor stent) could be advanced up the urethra.

In another dog, tumor compression caused collapse of the stent shortly after stent placement. Survival in the 17 dogs following successful stent placement ranged from 2-366 days (median 78 days). All 17 dogs received some form of medical therapy for the TCC after stent placement. The dog who died after 2 days had a severe adverse event to chemotherapy. A survey of the owners of dogs who had stents placed indicated that 16 of the 17 owners were satisfied with the outcome and would recommend stent placement to others. Although urethral stent placement is expected to be lifesaving in the vast majority of dogs, complications can occur. In the 19 dogs, complications included incontinence (39% of dogs), reobstruction from continued growth of urethral neoplasia (17%), acute reobstruction (5.5%), and stent migration (11%). Stent migration in 2 dogs was thought to be due to suboptimal stent size selection in one dog, and dramatic tumor shrink due to medical therapy in the other dog. The optimal tumor for successful stent placement appears to be one in which normal urethral tissue is present on either end of the tumor where the stent can be securely anchored. It is postulated that dogs with stents that only cover a small part of the urethra are less likely to experience incontinence than dogs with tumors requiring longer stents, but this has not been proven in studies to date. Similarly, the potential added risk of infection in dogs with stents has not been studied to date. It has been noted that extending the stent into the vagina should be avoided due to discomfort caused by the stent in that location.

In another report, the outcome of 42 dogs with obstructive carcinoma who underwent urethral stent (self-expanding metallic stent) placement was summarized. \(^{25}\) Relief of urethral obstruction was accomplished in 41 of 42 dogs (97.6%) dogs. In one dog, the stent migrated into the bladder after being deployed. Following stent placement, incontinence of varying severity occurred in 6 of 23 male and 5 of 19 female dogs. The median survival after stent placement was 78 days (range 7-536 days). Survival appeared longer (median 251 days, range 8-536 days) in the dogs treated with COX inhibitors before, and chemotherapy after, stent placement.

Another “non-surgical” approach reported to address urethral tumors is laser tumor resection. Pet owners may inquire about transurethral resection because they are aware of this approach in human bladder cancer patients. What most pet owners do not recognize, however, is this approach is most often applied to superficial tumors in humans where it is possible to resect the lesions without penetrating the bladder wall. Unfortunately, TCC in most dogs is invasive into the bladder wall. A procedure has been reported in which the investigators attempted to use ultrasound to guide endoscopic diode laser ablation of TCC. \(^{26}\) Using this and similar approaches, the main risk of laser resection of TCC in dogs remains bladder perforation. The value of laser resection has not been well defined as dogs in previous reports were receiving multi-modality treatment, and it is not possible to know which component(s) of the treatment could have been beneficial. At our institution, laser procedures are limited to carefully selected cases in which a “ball valve” type lesion clearly protruding into the lumen can be resected without substantial risk of perforation of the bladder or urethra.

**Progress in Bladder Cancer Management**

TCC has become a highly “treatable” cancer in dogs, and the outlook for dogs in regards to length and quality of life continues to improve. TCC, however, is rarely curable. Research in the prevention, early detection and intervention, and better therapies for TCC must continue. Tens of thousands of people also die from invasive bladder cancer worldwide each year. Experimentally-induced bladder tumors in animals for research do not sufficiently mimic the human condition. This means research in
dogs with naturally-occurring TCC is all the more important to improve the outlook for humans, as well as for dogs facing invasive bladder cancer.  


Radiotherapy for Lower Urinary Tract Tumors

Canine urogenital tumors present a therapeutic challenge. Curative surgery is often not an option because of tumor location. Traditional fractionated radiation therapy is associated with severe acute intestinal toxicity. Chemotherapy and palliative radiation therapy can improve clinical signs, but duration of response is limited. Limited information is available on novel techniques such as local chemotherapeutic infusions or photodynamic therapy. Recent technological advances in the fields of radiation oncology and minimally invasive surgery have provided promising new options for the treatment and management of these cancers. This lecture discuss focus on the application of specific technologies, such as intensity modulated radiation therapy (IMRT), image guided radiation therapy (IGRT), dynamic adaptive radiation therapy (DART) and stereotactic radiation therapy (SRT) for treatment of tumors of the bladder, prostate and urethra. We will also discuss management of radiation/tumor effects with stenting of the ureter and urethra.

Treatment of tumors of the bladder, urethra and prostate
Tumors of the lower urinary tract make up 2% of all canine cancers, and it is speculated that with the increased availability of ultrasound and computed tomography that clinical diagnosis is becoming more common. Transitional cell carcinoma of the bladder is the most frequently encountered tumor histologic type. Key to treating urinary tract tumors with radiation therapy is determining the location and extent of the tumor. The trigone of the bladder is the most common location although the entire bladder can be involved. Disease extension from the bladder to the urethra is seen in over 50% of patients while extension to the prostate occurs in almost 30% of male patients. Nodal (15%) and distant (14%) metastases can be present at initial presentation but is as high as 50% at the time of euthanasia. Therefore, accurate staging is of great importance. Confirmed or suspected regional lymph nodes should be treated. Other tumor types observed in this region include squamous cell carcinoma, adenocarcinoma, undifferentiated carcinoma, rhabdomyosarcoma, lymphoma, hemangiosarcoma, fibroma and other sarcomas. Tumor histology does not impact radiation prescription because radiation dose is always limited by normal tissue tolerance. However histologic type is extremely important in selecting the most appropriate post radiation chemotherapy regimen. Treatment with radiation therapy is technically challenging for a number of reasons. The bladder varies in size and location based not only on urine volume but on rectal content. (1) In addition, the target tissues move relative to the bony landmarks identifiable using standard port film techniques. Therefore when using a traditional radiation therapy technique such as three dimensional conformal radiation therapy (3D-CRT), the radiation treatment field necessary to include the tumor plus microscopic extension, (called the clinical target volume, or CTV) must be enlarged to take this variation into account.(2) This expanded radiation field is called the planning target volume (PTV). Adjacent normal tissue structures, such as the colon, rectum and intestines, are unnecessarily exposed to high doses of radiation, resulting in acute GI toxicity and consequential late effects. (3) This problem is exacerbated when disease has spread to regional nodes, further increasing the volume of tissue treated.
Technological Advances

Improvements in radiation oncology technology have vastly improved the ability to safely and effectively treat urinary tract tumors. **Intensity Modulated Radiation Therapy (IMRT)** controls the intensity of each beam through motorized collimator leaves (MLC) that move during treatment, allowing the dose to be sculpted around important normal structures. This technique requires a specific treatment planning system that uses **inverse planning**. Inverse planning requires that the various tumor structures (Gross tumor volume [GTV], CTV and PTV) as well as critical normal tissue structures be identified. This is achieved by outlining (contouring) each structure of interest on each slice of the CT scan. 

Dose objectives for each structure are entered, and a sophisticated algorithm attempts to meet all objectives. The 2 major benefits associated with IMRT are: 1) Dose to adjacent normal tissue structures can be minimized, dramatically reducing acute effects and 2) Radiation dose to the tumor can be increased, improving the probability of tumor control. Both of these benefits were realized when IMRT became the standard of care for prostatic tumors in men.

**Image guided radiation therapy (IGRT)** is another key component in the treatment of urinary tract tumors in dogs. IGRT utilizes imaging systems built into the therapeutic accelerator to confirm the location of the target immediately prior to treatment. For tumors that are firmly associated with boney structures, such as brain tumors and nasal tumors, orthogonal radiographs taken at treatment can be compared, using intrinsic software, to the digitally reconstructed radiographs (DRRs) from the treatment plan. For bladder and prostate cancers, a more advanced imaging technique, such as a cone beam CT (CBCT) is required. Prior to treatment, a CBCT is acquired and compared in 3 dimensions, to the CT used for the radiation treatment planning. IGRT allows the oncologist to confirm the location of the treatment target. The importance of this is that it allows a substantial decrease in the size of the PTV expansion, drastically decreasing the amount of normal tissues in the field.(1)

**Dynamic Adaptive Radiation Therapy (DART)** is a technique where the patient’s CBCT is evaluated each day before treatment to determine size/location of bladder. Based on previous work by Nieset (2), variations in bladder size/location can be predicted. With DART, a “small”, “medium” and “large” bladder plan is created.

**Intensity Modulated Image Guided Radiation Therapy (IG/IMRT)** combines the dose precision of IMRT with daily CBCT to minimize the PTV expansion. Twenty-one client owned dogs with tumors of the lower urinary tract were treated with IG/IMRT.(4) Primary tumors were located in the prostate (10), urinary bladder (9) or urethra (2). 54 to 58 Gy was administered in 20 daily fractions. Grade I and II GI toxicoses were observed in 33 and 5% of the dogs, respectively. Four dogs had late grad 3 GI or GU toxicosis. Median event-free survival was 317 days, and median survival was 654 days. The protocol was well tolerated and resulted in superior survival times compared to previous reports in the literature.

**Stereotactic Radiation Therapy (SRT)** is another new technology that may have applications for tumors limited to the prostate or urethra. It is unlikely that this technique can be safely applied to the bladder. Bladders are not tolerant of large doses per fraction and because of variations in size/location of bladders, it is difficult to target with the precision that is required for SRT to be successful.

SRT involves the use of high doses per fraction but overcomes the radiobiological limitations with stereotactically verified positioning and treatment delivery techniques that leave a minimal volume of normal tissue in the high dose area. Stereotactic radiation therapy, by definition, requires: 1) a tumor for targeting (not microscopic disease); 2) treatment planning and administration that will provide a dramatic dose drop off between the tumor and the surrounding normal tissue structures; and 3) a method of stereotactically verifying patient positioning. The result is that normal late responding tissue structures are spared through dose avoidance rather than by administering small doses per fraction.

The normal tissue structures still receive dose, and the dose per fraction is higher than for traditional radiation therapy. This technique is appealing because only 1-5 fractions of radiation are required
and treatments are generally well tolerated by the patient. Normal tissue tolerance for important regional late responding tissues have been well defined. (5) A major concern about SRT is that most canine urogenital tumors are transitional cell carcinomas. Unlike adenocarcinomas, these may not be restricted to the prostate. For tumors that seem restricted to prostate or urethra, we still recommend obtaining a cystoscopic biopsy of the bladder. the bladder even if it has a normal appearance.

Conclusions
The new renaissance in radiation oncology has introduced exciting technologies that may benefit canine and feline patients with urinary tract tumors. It is important that progress in this area be documented. Determining normal tissue tolerance of organs at risk is crucial to the development of optimal radiation therapy protocols. Minimally invasive procedures such as ureteral, urethral and rectal stents will become increasingly important as patients live longer and develop mild to moderate toxicities. As local tumor control improves, it is also critical that a chemotherapy protocols are carefully evaluated for efficacy.

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Oncologic “Surgery” of the Lower Urinary Tract

Classic surgery for cancer involving the urinary system is biopsy and extirpation. This lecture will discuss the traditional and the emerging technologies in treating or palliating cancer of the urinary system.

Laparoscopic-assisted cystotomy
To obtain a diagnostic sample in cases where less invasive means such as traumatic catheterization and cystoscopic biopsy are unsuccessful, a laparoscopic-assisted cystotomy may be indicated. In this approach, the urinary bladder is visualized with a laparoscope and, through a small opening in the body wall, the bladder surface is exteriorized and stabilized with stay sutures. A threaded trocar is placed into the bladder and a cystoscope is used to visualize the inside of the bladder and obtain biopsy samples. If the bladder is large enough, a 5 mm scope and alligator forceps or biopsy cup forceps may be used through the single port. Special care is taken to minimize urine spillage and to prevent contamination of the body wall with biopsy samples to prevent tumor seeding of the incision sites. To my knowledge, none of the tumor seeding cases described previously by Dr. Knapp occurred with minimally invasive surgery. When diagnosis is confirmed, medical therapy ensues.

Cystostomy tubes
In the past, we placed cystotomy tubes for palliative management of tumors involving the urethra, but these have been replaced by urethral stents.

Partial cystectomy
For small tumors of the bladder apex, partial cystectomy may be considered for removal of gross disease. However, many surgeons are hesitant to pursue this approach for fear of tumor regrowth from microscopic disease. When we attempt these operations, our goal is to obtain a 1 cm margin around the gross disease, and animals are placed on COX inhibitors postoperatively. The bladder is exteriorized through a caudal midline laparotomy and is packed off with laparotomy sponges. With the bladder distended with urine and the mass palpable at the apex, a series of stay sutures are placed in the bladder in grossly normal tissue at least 1 cm from the mass, further if possible. These stay sutures will then be used to handle the bladder reconstruction once the tumor is removed. The mass is then excised and the bladder is closed with a simple interrupted suture pattern. If possible, the suture line is then oversewn with an inverting Cushing's pattern. The bladder will be very small, and the animal will appear to be incontinent after surgery. These signs will improve with time as bladder capacity is restored.

When tumor invades or occludes the ureter, obstruction leads to hydronephrosis and impaired renal function. In the past, unilateral ureteral obstruction might have been treated by ureteronephrectomy. Today, our goal is to maintain function of both kidneys for as long as possible. Recently, techniques adapted from interventional urology have created additional options for treatment of local disease.

Ureteral stenting
TCC involving the trigone can be treated with ureteral stents. Prior to surgery, VD radiographs are obtained with a marker catheter to estimate the stent length that will be needed. The distance from the renal pelvis to the trigone is measured. Chose a stent that is longer than the measurement
because in most cases, the dilated ureter will have become tortuous and is much longer than what is measured. The stents can be placed by approaching the ureteral orifice from the bladder. In cases of extensive disease, it can be very difficult to identify the ureteral orifice and that precludes a cystoscopic approach. A cystotomy is then performed on the ventral surface of the bladder and is extensive to offer visualization of the trigone area. If a normal ureteral orifice can be identified, it is helpful to temporarily place a 3 fr red rubber feeding tube into that orifice to assist with determining the local of the opening of the abnormal ureter. A 0.035” floppy guide wire is used to probe the region to identify the ureteral opening. Once the ureter is accessed with the guide wire, a C-arm is then used to track the advancement of the guide wire to the renal pelvis. The urethral catheter is then advanced over the guide wire so that a coil remains in the renal pelvis as the guide wire is removed. C-arm images confirm the proper location of the ureteral stent.

For mass hard lesions involving the distal ureter that can’t be accessed from the bladder, one approach is to drill through the mass with a renal access needle from the ureter to inside the bladder and then pass the guide wire through the needle into the ureter. Although there is a potential concern for leakage of urine from the needle stick in the ureter, this was not an issue in the single case in which this approach was used. The ureter can also be approached by passing a renal trocar needle percutaneously into the renal pelvis using CT or US guidance. When the pelvis is accessed, a sample is withdrawn for culture, and a contrast study is performed. Through the needle, the guide wire is passed and directed caudally to the ureter. The guide wire is then advanced through the area of the ureteral obstruction to emerge inside the bladder. The needle is then removed. When combined with cystotomy, the ureteral stent is then passed over the guide wire and coiled in the renal pelvis. By creating access through the renal parenchyma, the risk of urine leakage is less.

In experienced hands, an IR approach may be used for antegrade placement of a ureteral stent. Renal access is obtained using a combination of US and CT guidance to place the renal trocar needle in the renal pelvis. A contrast study is performed and a guide wire is passed through the needle, down the ureter, into the bladder, and out the urethra to obtain “thru-and-thru” access. A sheath is passed retrograde over the guide wire to the renal pelvis and a second guide wire is then passed in a retrograde manner. The first guide wire maintains access at all times and the second guide wire is used for passage of the stent. After the stent is coiled in the renal pelvis, a push catheter is then used to deploy the distal coils in the urinary bladder. When the stent is properly placed, the thru-and-thru guide wire is removed. If disease extends to the urethra, urethral stenting can be performed in addition to the ureteral stenting, as discussed by Dr. Knapp.

Subcutaneous Ureteral Bypass (SUB)
An emerging therapy, initially developed for management of feline ureteral obstruction due to ureterolithiasis, is being used in selected cases for palliative management of ureteral obstruction due to tumor.

The SUB system is a combination of a nephrostomy tube and a cystostomy tube, connected via a port that is implanted in the subcutaneous tissues of the ventral abdominal wall. The system is placed surgically, using C-arm guidance. Access to the renal pelvis is obtained with a needle and the nephrostomy component is placed. A cuff on the outside of the catheter is secured to the surface of the kidney with surgical adhesive. Attention is then directed to the bladder and a purse-string suture is placed in the bladder and a cystostomy tube is placed via a needle in the center of the pursestring. The suture is tied, and a similar cuff is adhered to the surface of the bladder with surgical adhesive and 4 sutures. The tubing from the nephrostomy catheter and from the cystostomy tube are then tunneled through the body wall and connected to a port that is placed in the subcutaneous tissue. The port is secured to the external sheath of the rectus abdominis muscle with 4 sutures. Secure attachment is achieved with “boots” that are advanced over the outside of the catheter. Unilateral or bilateral SUBS can be placed. A port with 3 connectors is available to accept 2 nephrostomy tubes and 1 cystostomy tube. There has been limited experience with total extirpation of the bladder.
and placement of the “cystostomy tube” into the urethra. This renders the animal incontinent, but most animals with this stage of disease are incontinent already.

**IR Chemoembolization/Chemotherapy**

Interventional radiology techniques are being explored for local delivery of chemotherapy agents directly to tumors with the goal of delivering a much higher dose to the tumor that can be achieved with systemic chemotherapy. To access the prostate, Dr. Weisse uses vascular access of the femoral artery, a series of guide wires and microcatheters that are passed to directly access the blood supply to the tumor. Chemotherapy is delivered locally to the tumor. At this point, it is unknown whether combining the chemotherapy with beads or embolic therapy may be useful. And it is unknown whether it is better to access the blood supply from both the right and left arteries as a single or staged approach.

While some of these advanced surgical techniques may seem “extreme”, when combined with medical management, the animal’s life may be prolonged. When the quality of life is acceptable to the owner, these new therapies may represent options for continued management of tumors of the lower urinary tract.

Themed session
MELANOMA

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Melanoma: Behaviour and Biology

In dogs, tumors of melanocytes (cells that produce melanin) represent an extremely varied biological spectrum of disease, from the benign melanocytoma to the highly aggressive mucosal melanoma of the oral cavity. The varied presentation and biology often leads to confusion in diagnosis, prognosis (table 1) and treatment of this spectrum of diseases. Until we in veterinary oncology advance our understanding of basic genetic and molecular drivers of the varied types encountered, we will remain simplistic in our approach to a disease that requires a more individualized approach to treatment.

As in humans, there exist 3 general categories of melanoma: 1) Cutaneous melanoma, the most common form in humans and the second most common form in dogs, 2) Mucosal Melanoma, the most common malignant form in dogs is rare in people, and 3) Ocular melanoma, a relatively rare presentation in dogs and cats.

In humans, a significant majority of malignant melanomas are linked to exposure to ultraviolet rays from the sun. In dogs, most malignant melanomas occur in areas not exposed to sunlight and therefore the etiology is likely a combination of genetic and environmental factors unrelated to UV exposure.

In humans, alterations in cell signaling pathways are commonly implicated, in particular those of the MAP-kinase signaling pathway. BRAF and NRAS mutations are common in cutaneous melanoma in humans but are rare in mucosal melanoma. Similarly, in oral mucosal melanoma of dogs BRAF mutations have not been identified and NRAS mutations are rare as are PTEN mutations. C-kit mutations are quite common in humans with oral mucosal melanoma and while KIT expression is common in mucosal melanoma of dogs, activating c-kit mutations have not been clearly identified in canine mucosal melanomas. Most but not all oral mucosal melanomas in dogs have aggressive biology’s characterized by high metastatic rates and poor outcomes. Up to 10% of oral mucosal melanomas in dogs are benign (similar to blue nevi) and can be cured with surgery – this benign variety is typically smaller (< 2cm), deeply pigmented, with a low or absent mitotic index and lack surface ulceration.

Some excellent recent reviews and large compilations of canine melanomas with histologic and genetic characterization have recently been published (Simpson 2013, Gillard 2013, Smedley 2011, Esplin 2008) and have begun the much needed work of characterizing the genetic and pathologic subtypes of melanoma in dogs that is a necessary prerequisite to rational and more individualized treatment strategies for dogs with aggressive melanomas. Nearly all melanoma specimens from humans are subjected to genetic testing such that proper treatment recommendations can be made.
Table 1. Known or suspected prognostic factors identified in canine melanoma.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location</td>
<td>Tumors arising from haired skin are generally benign. Oral mucosal nail bed, footpad locations are generally malignant. Ocular melanomas of eyelid, limbal and uveal structures are usually benign.</td>
</tr>
<tr>
<td>Histology</td>
<td>Histologic criteria of malignant versus benign is predictive of biologic behavior. Determination based primarily on the mitotic index; however, nuclear atypia, lymphatic invasion and degree of pigmentation may also be prognostic.</td>
</tr>
<tr>
<td>Clinical stage</td>
<td>Visceral metastasis is a negative factor. Nodal metastasis is likely negative, however definitive data lacking.</td>
</tr>
<tr>
<td>Infiltration/invasion</td>
<td>Deep infiltration and bone lysis negative for oral melanoma and invasion beyond dermis is negative for cutaneous melanoma</td>
</tr>
<tr>
<td>Tumor cell proliferation rate</td>
<td>Immunohistochemical (e.g., MIB-1/Ki67, PCNA) techniques that measure proliferation shown to be prognostic. Only modestly more predictive than simple histology and mitotic index.</td>
</tr>
<tr>
<td>DNA ploidy</td>
<td>Prominent G2/M peaks predictive for malignant behavior. No more predictive than simple light microscopy, therefore not cost effective.</td>
</tr>
</tbody>
</table>


Smedley et al. (2011). Prognostic markers for canine melanocytic neoplasms: A comparative review of the literature and goals for future investigations
Malignant melanoma is the most common oral tumor in dogs and is associated with a high rate of regional and distant metastasis. (1) Tumor size is prognostic; regardless of treatment stage I tumors (≤ 2 cm) have better long term survival than larger tumors. Most dogs will succumb to metastatic disease. However, the local tumor will generally impact quality of life sooner than metastatic disease, so local disease should be addressed. Surgical excision and/or radiation therapy provide the best options for controlling loco-regional disease.

Thorough staging is recommended before treatment and should include CBC and platelet count, biochemical profile, urinalysis, thoracic radiographs (3 views), and local node assessment and cytology. Interestingly, nodes should be aspirated whether enlarged or normal in size; in a study of 100 dogs with melanoma, 40% had local nodal metastasis when not lymphadenomegally was present.(2) Abdominal ultrasound should be considered for evaluation of visceral metastasis.

Complete surgical excision is the most effective local treatment option, and there are a variety of innovative surgical options, depending on tumor location. Although “wide margins” are advocated, little outcome based information exists regarding surgical or radiation margin width. Wide margins of 2-3 cm have been advocated, but some surgeons feel that 1-2 cm is adequate. Rostral tumors may be diagnosed earlier than caudal tumors because they are easier seen by the client, improving the likelihood of complete excision. Caudal tumors may be larger and more invasive by the time of diagnosis, making surgery more challenging. Advanced imaging, such as CT, PET/CT or MRI as well as complete oral inspection should always be performed before excision of caudally located tumors so the extent of disease can be established and the surgery planned ahead of time. Incomplete excision of subclinical disease still provides a benefit to the patient, because radiation therapy is more effective when treating microscopic melanoma. The role of chemotherapy for gross of microscopic disease is unclear.

Radiation therapy has been used for many years for the treatment of oral melanoma.(3) Aggressive fractionated protocols have provided good tumor control, however their use must be weighed against the acute radiation effects and the probability of early metastatic spread. Currently, aggressive protocols are used for patients who have tumors with good prognostic indicators. In one study, 38 dogs with oral melanoma without evidence of metastasis were treated with 48 Gy delivered in 4 Gy fractions on a Monday-Wednesday-Friday schedule.(4) The median PFS was 17.8 months for all dogs and was stage dependent; for T1 tumors, it was 38 months; for T2 tumors, 11.7 months, and for T3 tumors, 12 months. Melanomas are believed to have a low α/β ratio, so higher doses of radiation per fraction are believed to improve response rates for melanoma. Coarsely fractionated protocols have more merit than for other types of oral tumors. A retrospective study of 140 dogs, most of which had regional or distant metastasis at presentation.(5) Coarsely fractionated (9 to 10 Gy weekly fractions to a total dose of 30 to 36 Gy) and conventionally fractionated (2 to 4 Gy fractions to as high as 45 Gy or more) protocols were used with or without surgery or chemotherapy.
The median times to first event and survival were 5 and 7 months, respectively. Tumor recurrence was the first event in only 27% of the dogs, with new metastases or death accounting for the other 63%. In 39 dogs with incompletely resected oral melanoma treated with coarsely fractionated radiation therapy plus platinum-based chemotherapy, the median survival time was 363 days.(6) The dogs received 6 weekly fractions of 6 Gy, with cisplatin (10 to 30 mg/m²) or carboplatin (90 mg/m²) administered 1 hour before irradiation. Fifteen percent of the dogs failed locally, and the median time to metastasis was 311 days. Palliative therapy can be used in the face of gross tumor. Bleeding and odor can be temporarily abated.

Treatment toxicity depends on the time-dose-fractionation of the protocol and the specific normal structures in the treatment field. For oral melanomas, this can include the skin, nasal cavity, and eyes. The major acute complication associated with radiation treatment of the oral cavity is mucositis. Mucositis always occurs to some degree in patients that have received irradiation to the oral cavity, pharynx, and/or esophagus. Mucositis typically begins during the second week of therapy and reaches a maximum severity during or shortly after the last week of therapy. Clinical signs include thickened saliva and tenderness of the mouth. These patients occasionally become reluctant to eat or drink and require supportive care. Low-salt foods are more palatable and less irritating to the oral mucosa than regular commercial diets. Oral mucositis should subside 1-2 weeks after therapy. Late complications of radiation specific to the oral cavity include, osteoradionecrosis, xerostomia and oronasal fistula development. Xerostomia (dryness of the mouth due to salivary dysfunction) is a common complication in human patients undergoing radiation therapy of the head and neck region. Osteoradionecrosis, or development of non-vital bone susceptible to pathologic fracture after radiotherapy, is an uncommon complication that can occur in any bone in a radiation field, typically years after treatment. The mandible is the most susceptible bone to osteoradionecrosis but as with most late effects, the risk can be minimized by administering radiation in lower dose per fraction. Oronasal fistula development after radiation is rare unless the hard or soft palate has been disrupted by an aggressive tumor or oral surgery.

Melanoma: New Treatment Strategies

The mainstay of local melanoma therapy in dogs remains surgical excision and/or radiation therapy. Depending on the location and malignant potential of a particular lesion, surgery alone can lead to cure or long-term control. However, a significant proportion of oral mucosal and subungual melanomas have metastatic potential and effective systemic therapies are essential for the management of biologically aggressive lesions. Unfortunately, malignant melanoma in dogs and humans is notoriously resistant to standard cytotoxic chemotherapy. Currently, considerable variability in reported progression-free and overall survival exist for dogs following excision and is consistent with the unpredictability of the disease with our current level of characterization. The varied outcomes reported combined with little correlative information regarding potential prognostic and predictive factors make outcome comparisons of surgery and additional adjuvant therapies difficult. Most canine therapeutic studies include insufficient numbers for adequate statistical power and fewer compare treatment protocols in a randomized prospective fashion. In addition, staging, inclusion and response criteria vary considerably between the treatment groups. Therefore, evaluations of efficacy between the various treatments are subject to bias, making direct comparisons difficult and indeed precarious.

Nearly all melanoma specimens from humans are subjected to genetic testing such that proper treatment recommendations can be made. Our understanding of the genetic and molecular players in canine melanoma is increasing but is still limited and is a necessary prerequisite to rational and more individualized treatment strategies for dogs with aggressive melanomas. Table 2 presents some of the current novel therapeutic approaches and potential therapeutic targets under investigation in veterinary oncology – these will be discussed during this presentation. Much of the activity currently involves immunomodulatory therapies designed to stimulate a clinically relevant immune response to melanoma. Vaccine technologies are a particularly active area of research, however we are still in relative infancy in our understanding of the immune system and the mechanisms by which tumors avoid immune attack. Vaccines strategies that are combined with the immunomodulation of tolerance such as CTLA4 inhibition (ipilimumab) and PD-1/PD-1L inhibitors are currently showing promise in human trials and will likely become important in the future in veterinary medicine.
Table 2. Novel therapeutic approaches/targets under evaluation for canine malignant melanoma either with in vitro and/or in vivo data

| Small molecule inhibitors | • NFκB inhibition (e.g., Bortezomib) – in vitro/vivo  
|                          | • Bcl-2 inhibition (e.g., ABT-737) – in vitro  
|                          | • Heat shock protein inhibition (e.g., STA-1474) – in vivo  
|                          | • NK-1R inhibition (e.g., maropitant) – in vitro/vivo  
|                          | • KIT inhibition (e.g., masitinab, toceranib) – in vitro/vivo  
|                          | • IGF-1, JAK1, PDGF inhibitors - in vitro/vivo  
|                          | • RACK1 – in vivo  
|                          | • AKT, mTOR - in vitro  

| Immunomodulation | • Nonspecific immunomodulators (e.g., L-MTP-PE, imiquimod IL-12, AdCD40L) – in vitro/vivo  
|                  | • Antitumor vaccines (e.g., DNA vaccines, whole cell vaccines, cytokine enhanced vaccines, electrogene transfer, dendritic cell vaccines) - in vitro/vivo  
|                  | • Radiation/immunomodulation combinations - in vitro/vivo  
|                  | • Methods of breaking tolerance – (e.g., Treg depletion, CTLA4 blockade, antiPD-1/PD-1L) - in vitro/vivo  

| siRNA, oncomirs (microRNA) | • siRNA targeting surviving – in vitro  
|                           | • anti-oncogenic microRNA tumor suppressors - in vitro  

| Oncolytic viruses | • Oncolytic vaccinia virus – in vitro  

| Cox-2 inhibitors | • in vitro/vivo  

| Other novel targets | • Leptin  
|                     | • Cadherins  
|                     | • Fascin-1  
|                     | • MMP9  
|                     | • Chondroitin sulfate proteoglycan-4  


Ito et al. (2013). The proteasome inhibitor bortezomib inhibits the growth of canine malignant melanoma cells in vitro and in vivo. *Veterinary J*, 198, 577-582.


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Comparative analysis of melanoma in dogs and humans: spontaneously occurring models for genetics and therapies of human melanoma

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Melanomas spontaneously occur in dogs, as in humans, due to genetic and environmental factors. The availability of dog breeds, presenting different melanoma type predispositions, considered as genetic isolates, should considerably simplify the search of the genetic causes of melanomas. To characterize their homologies and differences with human melanoma types, we obtained epidemiological data from 2350 melanocytic tumours from veterinary histopathology laboratories and we analyzed in depth epidemiological, clinical and histopathological data from 150 canine melanocytic tumours. We found that melanomas occur in the same anatomical locations than humans and interestingly, specific dog breeds present higher risk to develop oral or cutaneous melanoma, strongly reflecting genetic predispositions. We then proposed a histogenetic classification, based on the human classification and showed that canine melanomas appear good natural models for different human melanoma types, especially for the non-UV pathways of human melanomas.

We collected over 500 blood samples and 250 melanoma tumour /healthy samples from oral and cutaneous melanomas in high-risk dog breeds. Our first results show that oral melanomas present low frequency of somatic mutations in NRAS and PTEN genes, while no mutation have been found in the BRAF gene. The finding of mutations in the same genes as in human melanoma subtypes, and even, at the human hotspot sites, opens the field for specific targeted clinical trials in dogs. We expect genetic and epidemiological approaches to reveal genetic and environmental events involved in dog melanomas, considering each canine melanoma subtype in its predisposed breed as a piece of the complex human melanoma puzzle. Dogs thus represent invaluable models to identify novel genes, especially in the non-UV pathways, participating to the development of new therapies, which can benefit both human and veterinary medicine.
Comparative aspects of grey horse melanoma

In humans, melanomas are mostly aggressive melanocytic skin tumours, which metastasise to other organs and are usually lethal when left untreated at an early stage. In dogs, melanoma disease accounts for up to 7% of all skin tumours. Whilst lesions arising from haired skin are usually associated with a good prognosis, lesions affecting the oral cavity or mucocutaneous junctions show a high tendency for rapid progression and metastasis. Feline melanomas are rare, mainly involve the eye or eyelid and at these sites are behaviourally more malignant than dermal melanomas. A particular scenario is encountered in horses. Malignancy of the lesions rather seems to depend on the coat colour than the site of affection. In solid-coloured horses, melanomas are rare, yet show a highly aggressive behaviour. In contrast, grey horses frequently develop cutaneous melanomas and internal pigment cell tumours, especially during the second half of their life. It is estimated that up to 80% of grey horses older than 15 years develop melanomas. As a unique feature, grey horse melanomas are frequently encapsulated and thus poorly vascularised. As a consequence, metastasis of such lesions is retarded or inhibited. Gene analyses have allowed to assign the grey phenotype to a 350 kb region of equine chromosome 25. This region comprises the gene coding for syntaxin 17 (STX17). Southern blot analysis of non-grey versus grey horse DNA has revealed the presence of a 4.6 kb duplication in STX17 intron 6 of the latter. This duplication (D) was detected in both alleles of homozygous (D/D), yet only one allele of heterozygous grey horses (D/-), thus strongly suggesting a direct association of the duplication with the grey phenotype. Screening of 472 homozygous, and 255 heterozygous grey horses as well as 131 non-grey individuals fully confirmed this assumption. Remarkably, it could be shown that the incidence of melanoma significantly correlated with the D/D genotype, i.e. homozygous grey horses.

Currently, there is no universally effective therapy to cure melanoma disease. Treatment methods include the use of chemotherapeutics, radiotherapy, immune-modulators and gene expression suppressors. Difficulty to treat malignant melanoma in domestic animals and man is due to the fact that many aspects of disease initiation and progression still remain to be elucidated. An important step towards the development of an effective, targeted therapy against melanoma is the reliable identification of key regulatory factors in the progression of the disease. This can be achieved via gene expression profiling and mutation analyses. Microarray-based expression profiling has revealed novel pathways in the transformation of human melanocytes to malignant melanoma. DNA microarray technology is also used as a powerful predictive diagnostic tool for clinical applications. The European Organisation for Research and Treatment of Cancer (EORTC) has analysed 83 primary melanoma samples for a correlation between gene expression profile and clinical outcome. Using class comparison and prediction algorithms, a signature of 254 genes could be identified to act as an accurate indicator for 4-year distant metastasis-free survival. Another recent study used molecular profiling as a tool to predict the clinical outcome in patients suffering from stage III melanoma, which previously was not possible by any other means. For this, RNA was isolated from lymph node sections of 29 patients and subjected to oligonucleotide array analysis. A predictive score algorithm that was based on the expression of a subset of 21 genes could be accurately applied to distinguish patients with good from those with poor prognosis.

In horses, microarray technology has been used thus far to study the biological mechanisms involved...
in the development of common, papillomavirus-induced skin tumours termed sarcoids, revealing deregulated transcription of a broad range of genes, including MMP-1, TLR4, MHC-I, and CD68. Thanks to second generation-sequencing technologies, i.e. RNAseq, it has recently become feasible to quickly and inexpensively analyse transcriptomes, exomes, and complete genomes isolated from cancer samples.

We have used this method to analyse the transcriptomes of grey horse intact skin versus melanoma and primary melanoma cells derived from the same individuals. Methodologically, tumour and skin samples have been obtained with the owners’ written consent from affected grey horses and processed immediately. RNA has been isolated from collected tissue and cell material using an RNeasy Mini kit (Qiagen, Hilden, Germany). Then, a protocol that effectively amplifies non-ribosomal RNA molecules with lengths greater than 50 nucleotides (nts), including polyadenylated and non-polyadenylated transcripts, has been applied. Finally, RNAseq analysis was carried out by commercial partner BGI in Hong Kong.

Ongoing analysis of obtained data has so far revealed deregulated intratumoural transcription for over 2000 genes. Deregulated genes have been assigned to functional clusters, i.e. cell proliferation and apoptosis, angiogenesis, cell adhesion, motility and integrity, inflammation and immunity, as to determine deregulation-affected biological pathways. Genes with significantly upregulated transcription included e.g. melanoma marker genes that have already been identified in human melanoma, such as MAGE (melanoma antigen), MITF (microphthalmia-associated transcription factor), PMEL17 (aka Siv, gp100; pre-melanosome protein 17), or TYRP1 (tyrosinase-related protein 1). As already described for human and animal tumours, and most notably equine sarcoids, upregulation of MMP1 (matrix metalloproteinase 1) has been noted for equine melanoma tissue and cells. This is not surprising in so far as this proteinase lyses the extracellular matrix and thus promotes metastatic processes. Increased transcription has also been observed for collagen-degrading MMP9. Detected MMP1 and MMP9 overexpression has been confirmed on mRNA and protein level for additional melanoma (n=9), skin (n=8) and cell samples by quantitative RT-PCR, immunohistochemistry (IHC) and immunofluorescence (IF).

Particular attention is currently paid to the recorded tumour-associated overexpression of CD68 mRNA. CD68 is a glycoprotein that is typically expressed by monocytes/macrophages and thus regarded as specific marker for these cells. Accordingly, traditional immunological detection of monocytes/macrophages is carried out using anti-CD68 antibodies. Based on this type of assay, different human studies came to the conclusion that macrophages predominantly invade progressive cancers. Consequently, presence of tumour-associated macrophages, so called TAMs, is commonly correlated with poor prognosis.

On this basis, the observed upregulation of CD68 transcription in grey horse melanoma was initially attributed to the intratumoural presence of TAMs. However, it seemed rather absurd to correlate this finding with poor prognosis since the CD68-positive tumour material was mainly derived from semi-quiescent encapsulated nodules. Conventional RT-PCR from additional tissue and cell RNA material revealed the presence of CD68 transcripts in melanoma tissue and monocyte/macrophage-free primary equine melanoma cell lines. To verify this unexpected finding, we then analysed melanoma tissue sections by IHC. In addition, primary melanoma cell lines were analysed by IF using green fluorescently labelled anti-CD68 and red fluorescently labelled anti-melanoma (S100) antibodies. IHC revealed CD68-positive cells that were morphologically identified as tumour cells. IF showed co-localization of CD68 and S100 for a high percentage of tumour cells, providing conclusive evidence of equine melanoma cells expressing CD68 protein.

Although first descriptions of CD68 date from the 1980th, its function is still largely unknown. However, there are indications for CD68 having a key role in phagocytosis. This led us to assess the phagocytic potential of CD68+ primary melanoma cells. As shown by FACS analysis, these cells were able to engulf green fluorescently-labelled latex beads.

We hence conclude that grey horse melanoma cells express CD68 glycoprotein, and by this, are phagocytic cells. In depth investigations are required to elucidate the tumour biological impact of this phenomenon. Together with the finding of CD68 upregulation in equine sarcoid cells reported by
Prof. Nasir and her group (Glasgow, UK), our findings are indicative for CD68 representing a phagocytosis rather than a monocyte/macrophage marker. We hypothesize that CD68 is expressed by different types of cancer cells in different species. Our current work focusses on testing this hypothesis and investigating the pathobiological role of CD68 expression by cancer cells.

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Targeted Therapies in the Treatment of Human Cancers

The introduction of targeted therapies, especially monoclonal antibodies, have revolutionized the treatment of human cancer. When in the late 90’s trastuzumab as one of the first antibodies in the treatment of Her-2 positive breast cancer was introduced into the clinics (Hudis, 2007). Thus, a so far worse prognostic marker became a druggable target and improved progression as well as overall survival of the patients. Targeting tumor cells soon led to introduction of further antibodies against other members of the EGFR-family, such as cetuximab and panitumumab (Van et al., 2009; Douillard et al., 2010). Beside antibodies directly targeting tumor cells, the microenvironment has soon become the focus of therapeutic intervention. Thus, anti-VEGF targeting antibodies (Hurwitz et al., 2004) as well as genetic engineered anti-VEGF traps (Tabernero J, 2012) are in routine use for certain types of cancer such as those originating from colon, lung or kidney.

However, recent advances in our understanding of the intricate molecular mechanisms for malignant transformation and the aberrant control of complementary pathways have presented a much more complex set of challenges for the diagnostic and therapeutic disciplines than originally anticipated. Based on these advances, medical oncology has started to enter an era of individualized medicine where treatment selection for each cancer patient is becoming individualized or customized. For example, anti-EGFR antibodies in the treatment of colorectal cancer requires a genetic unaltered subsequent signaling pathway. A somatic RAS-gene mutation frequency of approximately 55% in all metastasized colorectal cancers prevents the application of a treatment option in this disease. Thus, mCRC requires a baseline gene mutations analysis to stratify the right patient for the right treatment. Notably, RAS-mutated mCRC patients, who received an anti-EGFR plus chemotherapy treatment, had a significant shorter progression-free survival as those patients who were treated with chemotherapy only (Douillard et al., 2013).

A tailored treatment is based on molecular and genetic characterization of the tumors including biomarker technology, which allow us to align the most appropriate treatment according to the patient’s disease. Although there is a general acceptance towards such an individualized approach thereby stratifying and subgrouping patients to improve the quality of clinical care in oncology, molecular profiling has just started to assist prediction.

Thus, several excellent demonstrations of the utility of prognostic and/or predictive biomarkers have emerged over the past decade. Currently introduced novel targeted agents in the treatment of cancer are approved for a certain subtype of cancer rather than for an individually diagnosed druggable target or for an entire signaling pathway. Therefore, there is increasing evidence that an extension of treatment protocols focusing
at molecular profile-based treatment decisions is needed thus generating a scientific rationale for an individualized treatment approach.


Tabernero J, V. C. E. L. R. e. a. (2012). Results From VELOUR, a Phase III Study of Aflibercept (A) Versus Placebo (pbo) in Combination with FOLFIRI for the Treatment of Patients (pt) with Previously Treated Metastatic Colorectal Cancer (MCRC). 2011 European Multidisciplinary Congress. Abstract 6LBA.

Oral presentations

The Wim Misdorp Award aims to encourage members in the earlier years of their career in veterinary oncology to pursue a period of scientific investigation in the field of veterinary oncology.

The award is to recognize outstanding contributions to the knowledge related to pathogenesis, diagnosis, therapy, prevention, or control of animal tumour-diseases. It is given once yearly at the ESVONC Annual General meeting to a veterinarian based upon his/her written abstract and oral presentation of scientific data at the meeting. In addition to a Certificate this award covers the travel and registration costs for the author to attend the Veterinary Cancer Society Annual Congress in the USA and to present their work.

The Wim Misdorp Award is sponsored by Novartis.
Definitive high-dose hypo-fractionated total pelvic irradiation with simultaneous boost in canine urinary CCT: a feasibility study and first clinical experiences

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Introduction

Lower urinary tract transitional cell carcinoma (CCT) pose challenge in order to the appropriate radiotherapy (RT) regimen. Organs at risk (OARs) within the irradiation field (ureters, rectum) are limiting factor in dose escalation. The primary aim of this study was to evaluate the technical feasibility of high-dose hypo-fractionated dynamic IMRT in not-resectable lower urinary CCT affected dogs. The secondary goal was to evaluate the toxicity and the efficacy of the RT regimens.

Materials and Methods

Three dogs with lower urinary tract CCT were treated with definitive high-dose hypo-fractionated RT with VMAT technique. Volume treatment definition include the gross tumor (GTV), the PTV1 (GTV+3mm), limphatics (PTV2), the entire bladder, prostate in males and uretra (PTV3), the entire pelvis except the rectal volume (PTV4). Dose prescriptions were 40 Gy to PTV1, 38 Gy to PTV2, 34 Gy to the PTV3, 30 Gy to the PTV4, in 6 fractions on alternate days. Piroxicam was subministered to all dogs. Serial clinical and CT/MRI examination were performed. Disease control and toxicity effects were evaluated according to RECIST and VRTOG criteria.

Results

Three T2N0M0 vesical CCT were treated. Prescription goals were obtained in all three cases with V95%>95% and V107%>2%. During follow up (mean 6 months) one partial response and two complete response were obtained. Two grade I cystitis developed. Non rectal toxicity was recognized.

Conclusions

Initial experiences with the RT regimen adopted indicate a feasibility and effectiveness in canine lower urinary canine CCT. Longer follow up and larger series are needed.
EXPRESSION OF CELL CYCLE REGULATORS PROTEINS (14-3-3σ & P53), AND VIMENTIN IN CANINE TRANSITIONAL CELL CARCINOMA OF THE URINARY BLADDER

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Introduction
Canine TCC shows striking similarity to human bladder cancer, however little is known of the molecules implicated in canine TCC. The aims of our study was to dilucidate if there are alterations in the expression of molecules especially intricated in cancer development as the tumor suppressors p53 and 14-3-3σ and also vimentin.

Materials and Methods
Nineteen canine bladder tumours cases and 2 normal bladder tissues were retrieved from our tissue archives. All samples had been fixed in 10% neutral buffered formalin and embedded in paraffin wax. Monoclonal antibodies against 14-3-3σ, p53 and vimentin and the EnVision™ immunohistochemical technique were employed. The reactions observed were semiquantitatively analyzed.

Results
Expression of 14-3-3σ was decreased in 53% of cases, p53 protein was over-expressed in 26% of cases and vimentin was neo-expressed in 21% of cases. Vascular invasion was observed in three cases and showed moderate to strong staining of both 14-3-3σ and vimentin.

Conclusions
The pattern of expression of 14-3-3σ indicates a probable role in the carcinogenesis and invasion mechanisms of canine TCC. The expression of vimentin in infiltrative cells reflexes the acquisition of an epithelial-mesenchimal transition that could lead to greater likelihood of metastasis. Finally, p53 appears to contribute to develop TCC with high frequency than previously thought.
Retrospective data analysis in a cohort of dogs with lower urinary tract tumours treated with advanced surgery

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Introduction
Surgery has a limited role in treatment of canine lower urinary tract tumours. In human medicine, invasive bladder transitional cell carcinoma (TCC) is treated by means of radical cystectomy followed, in most of the cases, by reconstruction with an ileal orthotopic neobladder. The aim of this study was to evaluate similar techniques in dogs.

Materials and Methods
Records of dogs with bladder and urethral TCCs diagnosed between 2005 and 2013 were identified. Dogs with histologic diagnosis of a primary lower urinary tract TCC treated with total or subtotal cystectomy or isolated urethrectomy were included. Surgical treatment consisted in urethrectomy and cystovaginal anastomosis (CVA), radical cystectomy and ureterovaginal anastomosis (UVA), radical cystectomy and ureterourethral anastomosis (UUA), and trigonal resection (TR).

Results
Eleven dogs were included. Nine purebred dogs and 2 cross breed dogs (10 females, one male), between 9 and 13 years of age (mean 10.2) were identified. Median body weight was 10.5 kg (range 4-37 kg). Three dogs were treated by CVA, 3 by UVA, 4 by UUA and 1 using TR. Three dogs received adjuvant chemotherapy. Survival time ranged between 7 days and 20 months (mean 6.45 months, median 3.5 months). Three dogs died for unrelated causes 14, 14 and 20 months after surgery respectively and one dog is still alive 6 months after surgery.

Conclusions
Our experience with complete cystectomy was good in terms of clinical outcome, although most of the patients died because of metastatic disease. Chemotherapy did not seem to improve survival in our cases.
Transcriptomic analysis identified up-regulation of solute carrier transporters and UDP glucuronosyltransferases in dogs with aggressive cutaneous mast cell tumors

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Introduction
The last decade has witnessed significant advances in personalized treatments as a result of genomic analysis and targeted drug therapies. In the present study, the transcriptome of dogs that had died because of mast cell tumors (MCTs) was characterized to identify a fingerprint having significant influence on cancer risk assessment, prognosis determination and treatment selection.

Materials and Methods
The transcriptome of 7 biopsies obtained from dogs with histologically-confirmed, surgically-removed MCT, treated with chemotherapy, and dead for MCT-related causes, was characterized by using Agilent Canine V2 4x44k DNA microarray, and compared with 40 samples obtained from dogs with histologically-confirmed, surgically-removed MCT that were alive after ~2 years of follow-up. Among the differentially expressed genes, 11 transcripts were chosen to validate DNA microarray results by qPCR and, then, their mRNA levels were measured in a cohort of 22 independent MCTs.

Results
Statistical analysis identified a total of 377 differentially expressed genes (FDR 5%). The functional annotation analysis provided evidence of drug metabolism, steroid metabolism and cell cycle pathways; particularly, members of solute carrier transporter (SLC) and UDP glucuronosyltransferase (UGT) gene families were identified as targets. The Principal Component Analysis of the 22 cohort MCTs identified two separate clusters, and one of these referred to 4 dogs that had died due to MCT.

Conclusions
SLCs and UGTs have been recently recognized in human cancer as important key factors in tumor progression and chemo-resistance. An in-depth analysis of their role in aggressive MCT might be useful in perspectives.

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The neutrophil to lymphocyte and albumin to globulin ratios as biomarkers predicting the histopathological grade of canine mast cell tumours

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Introduction
Canine mast cell tumours (MCTs) are variable in their biological behaviour and treatment decisions depend heavily on the histopathological grade. Biomarkers such as neutrophil to lymphocyte ratio (NLR) and albumin to globulin ratio (AGR) are used to predict biological behaviour of human neoplasms, but have not been widely studied in dogs.

Materials and Methods
A retrospective analysis of all dogs presenting to our hospital with MCTs from 2009-2014 was performed. Inclusion criteria were haematology and/or biochemistry results, a histopathological specimen and no steroid therapy or surgery within the last 60 days. Cutaneous MCTs were regraded if necessary using the two tier system proposed by Kiupel.

Results
Forty nine patients were included in the study (11 high grade, 38 low grade tumours). Receiver operator characteristic curve analyses was performed. These produced areas under the curve, representing the predictive ability of parameter, of 0.86 for NLR and 0.63 for AGR. The most suitable cut-off point for NLR was 8.7 (sensitivity 73%; specificity 97%). This value correctly classifying the largest proportion of tumours (92%). Neither AGR nor any individual parameter was found to have a useful a cut off value. NLR of low (5.59) and high grade (10.5) tumours were compared using a Mann-Whitney test which showed a significant difference between groups (p=0.0003).

Conclusions
An NLR cut-off of 8.7 can be used alongside existing prognostic tools (appearance, location etc.) to help to predict the likely grade of a canine mast cell tumour. This biomarker could therefore be used to guide treatment decisions prior to obtaining a histopathological diagnosis.
Comparison of Ki67 and mitotic index (MI) for predicting outcome in canine mast cell tumours (MCT)

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Introduction

Ki67 and MI scoring are independent predictors of survival in canine MCT, but often conflict making clinical decisions difficult. The study aims were to assess how closely they correlate and which more accurately predicts survival.

Materials and Methods

Dogs with MCT, with MI & Ki67 scoring, from three UK referral hospitals were retrospectively analysed. Ki67 analysis used the cut-off provided by the laboratory. Correlation of Ki67 and MI were assessed and Kaplan Meier survival analysis compared by Ki67 and MI scores.

Results

163 dogs were included. 57 dogs died, 37 due to MCT, 126 were censored (alive or dead of other causes). Using numeric Ki67 data with a quoted 1.8% cut-off (134), correlation between Ki67 and MI was good (Pearson’s coefficient = 0.758); agreement using Ki67 cut-offs was poor (Cohen’s kappa 2014 (MI≥5)). Using established reference values, high Ki67 was sensitive at predicting MCT related death (86.5%), but poorly specific (57.9%). MI≥5 was poorly sensitive (43.2%) but highly specific (94.4%). ROC analysis established MI≥2 as a superior cut-off, providing 75.7% sensitivity and 80.2% specificity. Ki67 produced a significant survival difference within the MI<2 group (p=0.009) but this is unlikely to be clinically significant. Ki67 score did not predict survival in tumours with MI≥2.

Conclusions

MI≥5 accurately predicted death but many dogs with MI<5 succumbed to MCT. Low Ki67 accurately predicted survival but high Ki67 was not necessarily a poor prognostic indicator. A lower MI cut-off (2) seemed more accurate, but created an unpredictable intermediate level (MI 2-5), where Ki67 did not provide further prognostic information.
Insights into Cox-2 dependent pathways in canine mast cell tumours: a role for microvascularization and tumoural proliferation

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Introduction
Mast cell tumours (MCTs) are among the most common cutaneous neoplasms in dogs and have a highly variable clinical behavior leading to the need of finding markers with potential as therapeutic targets. The overexpression of COX-2 seems to play a key role in MCTs, having being described an association between high Cox-2 immunoexpression and the histological grade of malignancy. In order to contribute to the understanding of the role of Cox-2 in MCTs, three main pathways were investigated: angiogenesis (by CD31 and VEGF expression), tumour cell proliferation (by Ki-67 antigen) and the inflammatory microenvironment (T-lymphocyte and macrophages).

Materials and Methods
Fifty (50) MCTs were included (15 grade I, 17 grade II and 18 grade III), and submitted to immuno-histochemical staining for detection of the following markers (COX-2, Ki-67, CD31, CD3 and MAC387).

Results
High COX-2 immunoexpression revealed a statistically significant association with histological grade (P = 0.002); CD31 (P = 0.0018); Ki-67 (P = 0.008); but not with macrophages and CD3+ T-lymphocytes. COX-2 was also positively correlated with CD31 (r = 0.334; P = 0.029) and Ki-67 proliferative index (r = 0.359; P = 0.011).

Conclusions
In this study, MCTs with high Cox-2 immunostaining presented predominantly an elevated histological grade of malignancy, a higher number of microvessels and a high proliferative index comparatively to tumours with low Cox-2 immunoexpression. Present results support the hypothesis that some of the underlying pathways involved in its biological aggressiveness including those related to angiogenesis and tumor proliferation might be related with COX-2-dependent mechanisms.
MicroRNA profiling of archival tumour biopsies for the discovery of new biomarkers for canine metastatic cutaneous mast cell tumours

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Introduction
Mast cell tumours (MCT) are the most common canine cutaneous malignancy. Histological grade and proliferative markers are used as prognostic indicators but limitations exist. MicroRNAs are post-transcriptional regulators of gene expression. They have key roles in all phases of cancer development and are dysregulated in numerous human cancers. The aim of this study was to evaluate microRNA profiling of cutaneous MCTs of dogs in order to potentially stratify patients with metastatic and non-metastatic disease.

Materials and Methods
Total RNA was isolated from 20 formalin-fixed, paraffin-embedded surgical biopsies of chemotherapy-naïve primary canine cutaneous MCTs with known outcome (10 metastatic and 10 non-metastatic). Metastatic patients had proven local and/or distant spread of disease. RNAs were labelled with Hy3 and hybridised to miRCURY LNA 7th generation microRNA (human, mouse & rat) microarrays. Background-subtracted hybridisation signal intensities were log2-transformed, and between array loess normalisation performed. MicroRNAs showing differences in expression between metastatic and non-metastatic MCTs were identified by T-test. P values were adjusted for multiple testing by application of a permutation test.

Results
Fourteen microRNAs displayed statistically significant differential expression (Pd increased expression in the metastatic MCTs). Reverse transcription-quantitative PCR was used to validate the differential expression of 6 of the 14 microRNAs which displayed the largest fold differences in expression.

Conclusions
This preliminary investigation suggests that microRNAs may be useful as markers for predicting the aggressive clinical behavior of cutaneous MCTs in dogs, and may indeed have a role in the regulation of canine MCT metastasis.
The evaluation of Progression Free Survival with masitinib incorporation into first line and rescue treatment protocols in 147 dogs with mast cell neoplasia

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Introduction
The goal of this retrospective study was to assess Progression Free Survival (PFS) of masitinib-incorporated treatment protocols in dogs with mast cell tumours (MCTs), co-analyzing tumour grade, metastasis status, remission rate and previous surgery.

Materials and Methods
Data (01/12/2008-01/01/2014) were collected from two veterinary oncology centres (NL&UK). MCTs, either surgically unmanageable, and/or with high metastatic risk or already metastasized were included and divided in cutaneous grade 1-2-3, subcutaneous (SQ) and other locations (OL). The first-line and rescue protocols masitinib-sole (MS), masitinib-prednisolone (MP), and masitinib-prednisolone-chemotherapy (MPC) were analyzed, using multivariate Cox-regression method. Adverse events were recorded according to VCOG-CTCAE v1.1.

Results
147 dogs were enrolled; 30 completed the intended treatment course (median/mean 186/241 days). Nine of twelve tumours checked, demonstrated c-kit mutations. Grade distributions per treatment group and median/mean PFS (days) were: MS 33gr2/17gr3/15SQ/2OL, median/mean PFS 112/304; MP 1gr1/25gr2/19gr3/8SQ/4OL, median/mean PFS 217/373; MPC 8gr2/5gr3/3SQ/2OL, median/mean PFS 49/202; Rescue 2gr2/2gr3/1SQ, median/mean PFS 38/71. Difference in PFS was significant for MS-MPC (P<0.001) and MP-MPC (P=0.0056), but not for MS-MP (P=0.118). This treatment effect was consistent at both centres, despite PFS differed significantly between them (P<0.001). PFS was significantly influenced by remission (P<0.001) and metastasis status (P<0.001), but not by grade or previous surgery. Severe adverse events included anaemia (n=3), hypoalbuminaemia (n=3), hepatopathy (n=3), gastrointestinal toxicity (n=2) and neutropenia (n=1).

Conclusions
Treatment protocol, completeness of remission and metastasis status, but not grade, were the most significant prognostic factors for PFS. Adding chemotherapy negatively influenced PFS. Impact of c-kit mutation could not be analysed as too few cases were evaluated.
A European multicentre pilot study to evaluate the combination of toceranib, lomustine and prednisolone for non-resectable or recurrent canine mast cell tumours

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Introduction
The goal of this prospective study is to assess the median survival time (MST) of a population of dogs with mast cell tumours (MCT), treated with a lomustine-toceranib-prednisolone protocol.

Materials and Methods
Dogs with measurable non-resectable, metastatic or recurrent grade II/III MCT, with a minimal estimated life expectancy of 8 weeks, were incorporated. Routine MCT staging was performed. Dogs were treated with lomustine at 70mg/m² (60-80) every three weeks and toceranib at 2.7mg/kg EOD. Prednisolone (1mg/kg EOD) was administered the day without toceranib. Response to treatment and treatment related toxicity were monitored according to RECIST 1.1 and VCOG-CTCAE v1.1, respectively. Tumour samples were analysed for c-kit status, Ki67, VEGFR expression levels utilizing immunohistochemistry.

Results
So far 12 dogs are included in the study and complete data are available for 8 dogs. Four dogs had multiple tumours, 4 dogs a single MCT. Response included 3 CR, 2 PR, 2 SD and 1 PD. Two patients (1 CR, 1 PR) are still alive (MST 432 days), 4 patients died due to different reasons, including PD and treatment related side effects (MST 147.8 days). Two patients were removed due to drug intolerability. Most common severe adverse events were neutropenia (n=9), vomiting (n=4), pyrexia (n=2), pancreatitis (n=1), and increased liver enzymes (n=1). Treatment delays and dose reductions were necessary in all 8 patients.

Conclusions
Treatment responses were encouraging. However, treatment related toxicities that resulted in multiple dose delays and reductions were frequent, indicating summation of common gastrointestinal and bone marrow associated side effects. Protocol modifications could improve tolerability.
Clinical relevance of simultaneous histopathological grading of canine cutaneous mast cell tumors at first presentation: a retrospective study on 386 cases

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Introduction
Historically, canine cutaneous mast cell tumors (MCTs) have been graded according to the Patnaik system. Recently, a new two-tier grading system was proposed. We retrospectively evaluated the clinical impact of both grading systems by taking into consideration clinical staging and outcome.

Materials and Methods
386 dogs with newly-diagnosed, histologically-confirmed MCTs undergoing complete staging were included for the investigation of clinical, histopathologic, therapeutic and survival data.

Results
All Patnaik grade 1 (P-G1) and Patnaik grade 3 (P-G3) MCTs were classified as Kiupel low-grade (K-LG) and Kiupel high-grade (K-HG) MCTs, respectively. Dogs with P-G1 and K-LG MCTs had a better outcome than dogs with P-G3 and K-HG MCTs, respectively. The majority of P-G2 MCTs were classified as K-LG tumors, and a small percentage (16.5%) as K-HG MCTs. Forty-four of 295 (14.9%) dogs with K-LG tumors had metastatic disease at presentation. When considering the combination P-G2/K-LG, 41/243 (16.9%) dogs had metastatic disease at presentation. Among dogs with P-G2 MCTs, the Kiupel grading system did not predict the development of metastases. A node-positive status was associated with a worse prognosis in the whole series as well as in the different subgroups, including P-G2/K-LG and P-G2/K-HG MCTs.

Conclusions
Patnaik and Kiupel grading systems are important parameters for the risk assessment in canine MCTs, and should be assessed in tandem. Histologic grading and nodal metastases equally contributed to anticipate the survival probability. The therapeutic management and prognosis of canine MCTs should not rely on the sole histologic grading, rather taking into account the clinical stage as well.
Radiotherapy in the treatment of 52 canine genitourinary carcinomas: preliminary results of a multi-institutional retrospective study

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Introduction
Therapy of canine genitourinary carcinomas has been challenging and limited to the use of Cox-2 inhibitors, chemotherapy and surgery. However treatment response and outcome remain poor. Patient referrals for radiotherapy are still rare and often limited to palliative care for advanced cases. This study attempts to describe the profile of cases, and the response and tolerance to various RT techniques, and associated survival.

Materials and Methods
This multi-institutional study evaluated retrospectively treatment outcomes of 52 dogs with macroscopic genitourinary carcinomas (bladder, urethral, prostate) that received megavoltage external beam RT (n=27) and HDR brachytherapy (n=25) between 2008 and 2013. Univariate, multivariate and survival analysis were performed.

Results
The treatments were well tolerated. The overall clinical response rate was 65.4%. OST was 231 days (n=52) and ST after radiotherapy was 195 days (n=49). ST was higher for dogs treated with RT doses >35Gy (208 vs. 81 days; p=0.028). Cases treated with adjuvant chemotherapy (n=32) had longer OST (317 vs. 122 days; p=0.005). Patients with regional metastases at diagnosis (n=9) had lower OST (59 vs. 239 days; p=0.000). Invading tumours also showed lower survival times (157 vs. 306 days; p=0.016). Long-term survival (>1 year) was achieved in 19.6% of cases.

Conclusions
These preliminary results confirmed the role of chemotherapy in genitourinary cancer patients. The clinical responses and treatment tolerance support the use of radiotherapy and suggest a better impact of higher doses protocols. Further recruitment is ongoing, yet later prospective studies are needed to define standard RT protocols for early stage and more advanced cases.
Response to toceranib in dogs with measurable, unresectable, recurrent or metastatic mammary carcinomas

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Introduction
Systemic therapy for non-resectable or metastatic canine mammary tumours is not well documented and there is little evidence of effective treatment options. Toceranib (Palladia®), a receptor tyrosine kinase inhibitor, was previously used in a Phase I trial, with a response for over 10 weeks in 4/5 canine mammary tumours.

Materials and Methods
Response to toceranib of measurable, recurrent or metastatic mammary carcinomas in dogs from four European countries was prospectively evaluated. Remission according to RECIST 1.1, adverse events, concurrent treatments, median treatment duration (TD), progression-free-interval (PFI) and quality of life survey were evaluated (QoL).

Results
Thirty-eight dogs were enrolled: 1CR, 8PR, 15SD and 14PD were reported. The median dose of toceranib was 2.98 mg/kg e.o.d. Side effects were reported in 40% of cases: gastroenteric (69%), haematologic (23%) and musculoskeletal (8%). Eight cases, 1 carcinosarcoma, 3 anaplastic carcinomas, 2 grade III simple carcinomas, 1 complex carcinoma and 1 carcinoma of unknown grade, showed a clinically relevant response with a duration of >10 weeks: 1CR, 4PR and 3SD. Median PFI and TD of these 8 dogs were 144 and 159 days, respectively. In the remaining 30 cases, there were 4 PR and 12 SD, with a mean PFI and TD of 26 and 28 days, respectively. PD was reported in 14 dogs. QoL under treatment was judged as good in most cases.

Conclusions
In 21% of dogs with advanced disease, a clinically relevant response was achieved, with a PFI of 144 days in comparison to 32 days for the non-responders.
Interrogating the Inflammasome in Canine Inflammatory Mammary Cancer

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Introduction
Human inflammatory breast cancer (IBC) and Canine inflammatory mammary cancer (IMC) are the most aggressive forms of mammary cancer. The clinical course is rapid and characterized by early metastasis and short survival times. Current research aims to establish new therapeutic targets to provide better treatment options. Here, we investigated protein and gene expression levels associated with the inflammatory microenvironment.

Materials and Methods
RNA was extracted from 32 formalin-fixed paraffin-embedded samples of canine mammary tumours (IMC=26; tubular carcinoma=6). The same tumour samples were processed and analysed for clinicopathological characteristics. cDNA was then obtained by Single Primer Isothermal Amplification technology and qPCR performed to measure relative gene expression levels of COX-2, Tribbles1, Synuclein Gamma (SNCG), Vimentin, CCL2, VEGF, CCR2 and CSF1R. Immunohistochemical (IHC) analysis of CCR2 and CSF1R expression was also performed. Statistical analysis was carried out using JMP - Statistical Analysis Software 5.0.1 and Statistical Package for the Social Sciences 17.0.

Results
Gene expression differences between IMC and non-IMC types were obtained for COX-2 (P=0.004), SNCG (P=0.006), Tribbles1 (P=0.025), VEGF (P=0.017) and CSF1R (P=0.045). Among these biomarkers correlations were found, particularly between SNCG and Tribbles1 (R=0.512, P=0.001). For IHC analysis, CCR2 expression was associated with presence of tumour emboli and a nuclear subcellular localization was observed predominantly in IMC tumours.

Conclusions
Upregulation of both SNCG and Tribbles1 in IMC might be the result of cooperation between the microenvironment and cancer cells, promoting efficient metastasis formation through epithelial to mesenchymal transition. However, further investigation is required to support this hypothesis.
THE PRESENCE OF THE SHORT FORM OF RON/STK TRANSCRIPT IS A PROGNOSTIC FACTOR OF POOR OUTCOME IN FELINE MAMMARY CARCINOMAS

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Introduction
RON/stk tyrosine kinase receptor, identified in cat as feline-stk, is activated by MSP and over-expressed in human breast cancer. Human RON gene is able to generate respectively the full length (fl) and the short forms (sf) of the transcripts. sf-RON is generated from an alternative transcriptional start from a second promoter within the intron 10 which preserves the kinase activity of the receptor. The aim of this research was to identify the presence of the sf-RON in feline mammary tumors (FMCs) and evaluate the expression of sf-RON and fl-RON in FMCs in relation to clinical follow-up and histological grading.

Materials and Methods
Immunohistochemical expression of RON and MSP was evaluated on 50 FMCs. To detect sf-RON, RNA was extracted from 47 FMCs and RT-PCR with primers annealing on exon 10 and exon 11 was performed on cDNA.

Results
Immunohistochemical expression of RON and MSP was observed in the 68% and 58% of FMCs respectively while the 52% of the cases co-expressed both proteins. The feline short form of RON was detected in 27/47 FMCs and resulted statistically associated with the poorly differentiated histological grade. Queens with FMC showing the sf-RON had a shorter disease free period and a shorter survival if compared with patients negative for sf-RON. Expression of RON, MSP or both in FMC was not correlated with clinical outcome.

Conclusions
The presence of the sf-RON in feline specie was firstly identified and only this isoform represents a prognostic factor of poor outcome in feline mammary carcinomas.
Gadolinium neutron capture therapy for III stage malignant oral melanoma in 34 dogs

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Introduction
Neutron capture therapy (NCT) is an experimental treatment used for human patients with head and neck cancer, particularly, glioblastoma and metastatic melanoma [Yasui L.S. et al., 2008; Barth R.F. et al., 2012]. Gadolinium is a potential agent for NCT as a nuclear capture reaction occurs when Gd-157 is irradiated with low energy thermal neutrons. The release of focal high-dose radiation induces chromosome aberrations. In vitro studies show effectiveness of Gd-NCT in inducing cell death [Franken N.A. et al., 2006]. The study was undertaken to assess efficacy and adverse effects of Gd-NCT in 34 dogs with oral malignant melanoma (OMM).

Materials and Methods
34 dogs with III stage OMM were treated with Gd-NCT. Contrast agent Gd-DTPA was administered intratumoral immediately prior to irradiation. NCT was performed at the IRT MEPhI reactor with neutron beam characteristics as described by Mitin V.N. et al. (2009). In dogs with confirmed regional lymph node metastasis lymphadenectomy was also performed. Response rates and overall survival time were recorded. Adverse effects after treatment were also noted.

Results
Gd-NCT induced partial response in 50% (17/34) of dogs and complete response in 47% (16/34). The overall survival time calculated using Kaplan-Meier method was 204.2 days. Common adverse effects were skin depigmentation (13/34), alopecia (10/34) and radiation-induced stomatitis and dermatitis (10/34), in rare cases – radiation necrosis of skin and soft tissues (5/34) and conjunctivitis (2/34).

Conclusions
Gd-NCT showed promising results in dogs with oral malignant melanoma and needs further investigation.
An open-label phase 1 dose-escalation clinical trial to determine the maximally tolerated dose and
dose-limiting toxicities of a single intravenous gemcitabine administration in dogs with advanced
solid tumors

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Introduction
Gemcitabine is a nucleoside analogue with unique metabolic and mechanistic properties. Preliminary
studies in dogs have shown a mild, schedule-dependent toxic profile with a broad range of
gemcitabine dosages (350-800 mg/m²). We determined maximally tolerated dose (MTD), dose-
limiting toxicity (DLT), pharmacokinetics, and preliminary antitumor activity of intravenous
gemcitabine in dogs with advanced solid tumors.

Materials and Methods
Dogs with advanced cancer were enrolled in an open-label phase 1 study of 30-min intravenous
gemcitabine infusion. Gemcitabine was administered starting at 800 mg/m² using dose escalation of
50 mg/m² increments with 3 dogs per dose level. MTD was established based on the number of dogs
experiencing a DLT after one cycle. Additional dogs were enrolled at MTD to better characterize
tolerability and pharmacokinetics. Treatment continued until disease progression or unacceptable
toxicity.

Results
Twenty dogs were enrolled and treated at 4 dose levels, ranging from 800 mg/m² to 950 mg/m².
Neutropenia was identified as the dose-limiting toxic event. MTD was 900 mg/m². DLT was observed
at 950 mg/m² in 2 dogs, and consisted of grade 4 febrile neutropenia. There were no non-
hematological DLTs. Eighteen dogs received multiple doses of gemcitabine, and none of them
experienced severe toxicity from any of their subsequent treatments. At 900 mg/m² dose level, 5
complete responses and 5 partial responses were observed.

Conclusions
The recommended dose of gemcitabine for future phase 2 studies is weekly 900 mg/m². In dogs with
advanced solid tumor this dose level is well tolerated and merits further evaluation.
Outcome in Dogs with Stage IIIb Anal Sac Adenocarcinoma Treated with Coarse Fractionated Radiation Therapy

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Introduction
Surgery in stage IIIb anal sac adenocarcinoma (ASAC; any T, N>4.5cm) is often not feasible or declined by the owner although clinical symptoms can be life-threatening. Palliative radiation therapy (RT) might offer a valuable treatment option.

Materials and Methods
Dogs with stage IIIb ASAC treated with a 6-MV linac and computerized 3D treatment planning (3D-conformal or IMRT) and 8x3.8 Gy (total dose 30.4 Gy) were included. Confining factors such as chemotherapy or surgery were evaluated for relevance in progression-free interval (PFI) and overall survival (OS).

Results
All 14 dogs presented with a palpable mass. 8/14 had defecation problems of which 3 were life-threatening obstipation, 3/14 dogs were hypercalcemic. In 29% prior surgery was performed, however at presentation all dogs had stage IIIb-disease with a mean gross tumor volume (GTV) of 166cm³. In addition to RT, 43% received (neo)adjuvant chemotherapy. All dogs showed partial/complete resolution of defecation problems. Median follow-up was 315 days, median clinical PFI 317 days (95% CI: 259-375), and median OS 319 days (95% CI: 227-411). 4 dogs are still alive, 3/10 dogs died of causes other than ASAC. Acute radiation toxicity was limited to grade 2 colitis, no late effects were seen. PFI was neither influenced by GTV, hypercalcemia, nor chemotherapy; OS was not influenced by GTV nor hypercalcemia, but shorter in cases receiving chemotherapy.

Conclusions
Hypofractionated RT is well tolerated and a valid treatment option in large ASAC. Although true palliative RT is defined as sole pain/symptom relief, the present results suggest prolonged locoregional disease stabilization in the majority of dogs.
Clinical outcome, IHC features and potential treatment targets in feline histiocytic disorders: a retrospective, multinational study

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Introduction
Histiocytic disease is rare in cats. Our aim was to document clinical findings and outcome in feline histiocytic disorders, and to identify potential treatment targets. PDGFrβ expression has been demonstrated in human histiocytic diseases that responded to tyrosine kinase inhibitors (TKIs), and TKIs that target this molecule and CD117 are available in veterinary medicine (masitinib and toceranib).

Materials and Methods
Samples from cats with confirmed histiocytic disorders were reviewed and characterised by immunohistochemistry (CD18, MHC II, leukocyte markers, toluidine blue, C-Kit and PDGFrβ). Clinical presentation, treatment and outcome were evaluated.

Results
Fifteen cats were enrolled (two were excluded). Five feline progressive histiocytosis (FPH) cases, 6 histiocytic sarcomas (HS) and 2 haemophagocytic syndromes were identified. C-kit was negative in all cases. Approximately half showed high PDGFrβ expression and half low. All FPHs highly expressed PDGFrβ. Low PDGFrβ appeared correlated with shorter survival. Treatments included surgery, corticosteroids, lomustine, TKIs and radiotherapy. Survival times ranged from 7 to 515 days, with 5 patients still alive 1-3 years after the diagnosis. Due to low numbers, it was difficult to correlate treatment modalities with outcome. However, partial responses were recorded in measurable disease with masitinib, toceranib and lomustine, and radiotherapy achieved long term control in some cases.

Conclusions
The prognosis for feline histiocytic disease is guarded, though localised HS may be more manageable. PDGFrβ, but not CD117, may represent a therapeutic target. Radiotherapy in combination with lomustine can achieve long-term survival in localised HS.
ASSESSMENT OF INFILTRATING LYMPHOCYTES IN HISTIOCYTIC SARCOMA OF THE FLAT COATED RETRIEVER: A COMPARISON OF DIFFERENT LOCATIONS

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Introduction
The Flat-coated retriever is predisposed to development of poorly differentiated, highly malignant sarcomas, classified as histiocytic sarcoma, with an immunophenotype consistent with myeloid dendritic antigen presenting cell origin: CD1+, CD4-, CD11c+, CD11d-, MHC II+, ICAM-1+, Thy-1+. The cellular origin of canine histiocytic sarcomas remains unknown. This tumour typically arises in the deep musculature of the limbs, splenic and disseminated form are also recognised. The purpose of the present study was to establish the presence of Tregs in histiocytic sarcoma of FCR, and to assess possible differences in their distribution between different locations.

Materials and Methods
Forty samples of histiocytic sarcoma were examined. Diagnosis was confirmed by H&E and positive immunostaining with MHC II and CD18. Three micron sections were cut and stained with CD3, CD79, CD25, CD45, Foxp3. Five fields for each slide were examined and the percentage and strength of staining was assessed and scored.

Results
Of the 40 samples, 15 originated in spleen, 11 limb and 14 „other location”. All tumours had a 10 - 20% infiltrate of CD3 positive cells. Foxp3 was expressed in tumours from all three locations although the percentage Foxp3 expression was significantly higher in tumours from the limb, whereas expression of CD45 was higher in tumours from the spleen.

Conclusions
The significant difference in expression of Foxp3 and CD45 in histiocytic sarcomas originating in different locations may provide a valuable insight into the difference in tumour microenvironment. Further work is needed to better evaluate histiocytic sarcoma’s microenvironment by looking at fresh tissue and assessing other factors & cells of the immune system in the tumour microenvironment.
Gene expression profiling in localized and disseminated histiocytic sarcomas in the predisposed flatcoated retriever dog

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Introduction
Exploring the downside of canine inbreeding strategies, the soft tissue- and the visceral form, we aim to determine if there are differences in gene expression within these two common subtypes of histiocytic sarcomas, and furthermore whether we can identify common differences in gene expression of histiocytic malignancies when comparing with healthy spleen.

Materials and Methods
Microarray analysis and pathway analyses were performed on fresh-frozen tissues obtained from Flatcoated retrievers with localized, soft tissue histiocytic sarcomas (STHS) and disseminated, visceral histiocytic sarcomas (VHS) and on normal canine spleens from various breeds. Expression differences of several genes were validated with quantitative real-time PCR (qPCR) analyses.

Results
When comparing both the soft tissue and the visceral form with healthy spleen, QPCR analyses identified the significantly altered expression of nine genes; PPBP, SpiC, VCAM1, ENPEP, ITGAD (down-regulated), and GTSF1, Col3a1, CD90 and LUM (up-regulated). QPCR analyses could also confirm the significantly aberrant expression of three genes when comparing STHS and VHS; C6 was up-regulated; CLEC12A and CCL5 were down-regulated in the visceral histiocytic sarcoma compared to the soft tissue form.

Conclusions
At the comparison of histiocytic malignancies as a group with healthy spleen, this study was able to identify altered expression of several genes that were not yet implicated in histiocytic sarcoma manifestations in the dog. Also, this study found some variations in gene expression between the two forms of histiocytic malignancies.
Identification of Neuroendocrine Pancreatic Cancer Stem Cells

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Introduction
Cancer stem cells (CSCs) are key target in developing novel anti-cancer therapeutics, since CSCs are responsible for initiating metastasis, and for the resistance to chemotherapy and radiation. Currently, there is only limited evidence of the existence of CSCs in pancreatic neuroendocrine tumours (NETs) and in its subgroup insulinomas (INS), which are rare tumour types. The focus of this study was to develop enrichment methods for selection of CSCs and to find subgroups of cells, within the heterogenous population of pancreatic NET cells, displaying specific CSC characteristics.

Materials and Methods
Sphere culture assays and doxorubicin cytotoxicity assays were developed and used to enrich an INS (CM) and a pancreatic carcinoid (BON1) cell line for CSCs. Gene expression of stem cell genes and surface markers was measured by quantitative real-time polymerase chain reactions (qPCR). A cluster analysis was performed to assess expression linkage between the different culture assays.

Results
Upregulation of stem cell markers CD34, CD45, CD90 and Oct4 was seen in doxorubicin resistant cells. Cultured tumourspheres showed higher mRNA expression of surface markers CD45, CD133 and CD227 compared to monolayer cells. Cluster analysis showed closer gene expression linkage between enrichment methods compared to cell line identities.

Conclusion
This research shows two methods of enrichment for CSC-like cells in pancreatic NETs and characterises the gene expression pattern in these CSC-like cells. This study provides an in vitro non-adherent culture system to grow pancreatic NET tumourspheres.

Winner Basic Science Award Dutch Animal Cancer Foundation
Response of canine intranasal tumors to a COX-2 inhibitor (Meloxicam) alone, and in combination with hypofractionated radiation therapy

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Introduction
Radiation therapy with curative or palliative intent is regarded standard of care in dogs with intranasal neoplasias. The majority of intranasal carcinomas express COX-2. The goal of this prospective study was to evaluate the response of canine intranasal tumors to a COX-2 inhibitor (Meloxicam) alone and in combination with palliative radiation therapy.

Materials and Methods
25 dogs with intranasal tumors (15 carcinomas, 9 sarcomas, 1 aesthesioneuroblastoma) were treated with Meloxicam for 2-3 weeks followed by palliative hypofractionated radiation therapy (3 x 8 Gy). Response to therapy was determined by CT-based measurement of tumor volume. COX-2 expression was determined by immunohistochemistry.

Results
84% of all nasal tumors (13/15 carcinomas, 7/9 sarcomas and the aesthesioneuroblastoma) stained positive for COX-2 expression. After Meloxicam therapy a partial remission (PR) by an average of 36.4% (range, 12-75%) was observed in 5 (33%) of the 15 patients with carcinomas, but there was no association with the degree of COX-2 expression. Following radiation, 11/25 (44%) of the patients showed a >50% remission of their tumors, including all 5 Meloxicam-responders. The median PFI for all dogs was 147 days (0-545d), median ST was 214 days (57-1828d). Tumor stage was significantly associated with ST (p ≤0.010). Dogs with carcinomas with a PR on combined Meloxicam-radiation therapy had a significantly longer median ST compared to dogs with carcinomas that did not respond (285d vs 107d; p=0.02).

Conclusions
Therapy with Meloxicam can lead to partial tumor remissions in dogs with intranasal carcinomas. Responders have significantly improved ST after palliative radiation compared to patients without response.
Hematologic abnormalities in canine diffuse large B cell lymphoma: what we have found in a group of 37 dogs

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Introduction
In dogs the most common WHO entity seems to be Diffuse Large B cell lymphoma. Studies evaluating recent WHO lymphoma entities treatment response, as well as prognostic markers are lacking.

Materials and Methods
Thirthy seven cases of canine diffuse large B cell lymphoma (cDLBCL) treated with a 19 week CHOP protocol were analysed for haematological abnormalities at presentation. Hematological data was obtained with an in-house multi-parameter counter and results confirmed by microscopic evaluation of stained blood smears. Survival analysis using Kaplan-Meier test was performed for each different observed abnormalities.

Results
Sixty seven percent of dogs had anemia at presentation, 24% had trombocytopenia and 30% lymphopenia. Neutrophil:Lymphocyte ratio was below the reference value on 30% of dogs. Median survival time for the group was 253 days. Patients with anemia and monocytopenia had a MST of 81 days, and 51 days, respectively. For Neutrophil:Lymphocyte ratio, patients with result >3.0 had MST of 103 days.

Conclusions
Aneamia and lymphopenia are the most common findings and are associated with lower MST in dogs with DLBCL. This data may indicate a possible prognostic relevance of these parameters for cDLBCL. Monocytopenia, a new identified abnormality was strongly associated with shorter MST as well as Neutrophil:Lymphocyte ratio higher than 3.0. Despite our statistical relevant data, further, prognostic, multicentric studies are required to strongly evaluate wich haematological abnormalities can have prognostic value.
ABC-transporter expression in canine multicentric lymphoma

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Introduction
Canine multicentric lymphoma (CML) is routinely treated with a multidrug chemotherapy protocol. Although initially successful, tumor recurrence is common and often refractory to treatment. Failure to respond to chemotherapy is thought to result from drug resistance and associated with active efflux of cytostatic drugs by ABC-transporter proteins. The goal of the current study was to characterize ABC-transporter expression in CML and assess their use as a prognostic factor.

Materials and Methods
Lymph node aspirates were collected from 64 dogs with CML treated with a standardized doxorubicin-based chemotherapy protocol. ABCB1, -B5, -B8, -C1, -C3, and -G2 mRNA expression (qPCR) were measured and related to clinical data and treatment outcome.

Results
No significant correlations were found between ABC-transporter expression and sex, age, weight, stage or substage. T-cell lymphoma and hypercalcemia were associated with a higher ABCB5- (3.91 fold, P = 0.08; 11.34 fold, P = 0.03) and ABCC5-expression (3.50 fold, P = 0.001; 10.50 fold, P < 0.001), and lower ABCC1-expression (0.32 fold, P < 0.001; 0.30 fold, P = 0.01) than B-cell lymphomas and normocalcemia. Expression of ABC-transporters was not significantly correlated with length of first DFP or OS. Expression of the various ABC-transporters was strongly correlated suggesting three distinct groups of ABC-transporters, as was confirmed with an unsupervised hierarchical cluster analysis.

Conclusions
ABC-transporter mRNA was detected in all CML samples and a significant difference in expression was found between T- and B-cell lymphomas, potentially explaining the difference in response to a CHOP-base protocol. Unexpectedly, ABC-transporter mRNA expression was not prognostic for DFP or OS.
PROGNOSTIC SIGNIFICANCE OF KI67 EVALUATED BY FLOW CYTOMETRY IN DOGS WITH HIGH GRADE B-CELL LYMPHOMA

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Introduction
Ki67 is associated with tumor grading and prognosis in different subtypes of human non-Hodgkin lymphoma. We recently reported that flow cytometric detection of Ki67 is useful in discriminating between high and low grade canine lymphomas, but its reliability as prognostic marker is still unclear. Aim of the study was to assess the prognostic significance of Ki67 in dogs with high grade B-cell lymphoma (HGB-LSA).

Materials and Methods
45 HGB-LSAs treated with Wisconsin-Madison protocol were included. Diagnosis was based on flow cytometric immunophenotyping and cytology (updated Kiel classification). Ki67-positive cells (Ki67%) were determined by flow cytometry on lymph node aspirates using FITC-conjugated monoclonal antibody (clone MIB-1). Following variables were investigated for association with overall survival (OS) and relapse free interval (RFI) using univariate, multivariate and survival (Kaplan-Meyer) analyses: Ki67% (≤20%, 20.1-40%, >40%), breed (purebred or crossbred), sex (male or female), age (< or ≥ 10yrs), stage (I-IV or V), substage (a or b), complete remission (CR, yes or no).

Results
On multivariate analyses Ki67% (P=0.036) and achievement of CR (P=0.009) were independent prognostic factors for OS, while none of the considered variables reached significance for RFI. Dogs with Ki67=20.1-40% presented significantly longer OS and RFI (median=593 and 406 days, respectively) than dogs with Ki67≤20% (median=177 and 200 days) and Ki67%>40 (median= 258 and 142 days).

Conclusions
Ki67 is an independent predictor of OS in treated HGB-LSAs; intermediate values are associated with best prognosis. We suggest flow cytometric determination of Ki67 as a diagnostic and prognostic tool to improve clinical usefulness of cytology and immunophenotyping.
**P-H2AX as a marker of genomic instability in dog with lymphoma undergoing chemotherapy: preliminary study**

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**Introduction**

H2A is one of the component of the histone octamer that forms the nucleosome. The isoform H2AX represents 2 to 25% of the total histone H2A expressed by cells and is implicated in the genomic response to DNA damage. Phosphorylated H2AX (P-H2AX) accumulates at DNA double strand breaks, where it assists to the recruitment of DNA repair and signaling factors. Therefore, induction of P-H2AX is a reliable marker of DNA damage. We hypothesize that P-H2AX could identify early genomic damage in lymphoma cells of dogs undergoing chemotherapy with Doxorubicin, an information that is not provided by markers of cellular proliferation, such as Ki-67.

**Materials and Methods**

Dogs presented at the Oncovet center, in which naive multicentric high-grade lymphoma was identified, were prospectively recruited. Chemotherapy (CT) with doxorubicin was initiated and serial lymph node biopsies and fine needle aspiration (FNA) were performed before chemotherapy and 2 to 6 hours post-CT. Lymph node tissues were used for measurement of Ki-67 expression, whereas FNA samples were used for measurement of P-H2AX (using flow cytometry method).

**Results**

Eight cases were prospectively recruited, of which 6 provide adequate samples for measurement of P-H2AX. A marked increase in the expression of P-H2AX was observed 2 and 4 hours post-CT, whereas Ki-67 expression was not modified following treatment.

**Conclusions**

Assessment of P-H2AX on FNA of lymph node is feasible in dogs with lymphoma, and allows early assessment of DNA lesion. This biomarker could be used in prospective clinical studies focusing on the early detection of tumoral cell resistance to treatment.
Progression Free Survival of dogs with high-grade T-cell lymphoma, treated with L-CHOP or CCNU-L-CHOP-based protocols as first-line therapy

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Introduction

High-grade T-cell lymphoma (HGTCL) in dogs is associated with a worse prognosis compared to high-grade B-cell phenotypes. Independent of the immune-phenotype, most dogs with high-grade lymphoma are treated with a (L)-CHOP protocol, while lomustine (CCNU) is reserved as treatment for therapy-resistant high-grade lymphomas. The purpose of this retrospective study was to compare Progression Free Survival (PFS) of dogs with HGTCL treated with first-line (L)-CHOP or CCNU-(L)-CHOP protocols.

Materials and Methods

Records of two veterinary oncology referral centers were reviewed. Included were dogs with HGTCL, first-line treated with either (L)-CHOP or CCNU-(L)-CHOP-based protocols. Kaplan-Meier method and log-rank test were used for survival analysis. Adverse events were documented according VCOG-CTCAE v1.1.

Results

Forty-two dogs were included. Twenty received (L)-CHOP, 17 CCNU-(L)-CHOP and 5 CCNU-prednisolone. In the (L)-CHOP group 45% achieved CR, 20% PR, 25% SD and 10% PD. In the CCNU-(L)-CHOP group 82.3% achieved CR, 11.8% PR and 5.9% SD. These differences were, however, not significant. PFS for (L)-CHOP and CCNU-(L)-CHOP differed significantly (P=0.005), with a median of 49 and 243 days, respectively. In the CCNU-prednisolone group CR was 40%, with a median PFS of 239 days. No significant adverse events were noticed in the (L)-CHOP group. In the CCNU-based group, grade 2-3 renal toxicity (n=11), grade 1-3 neutropenia (n=10), grade 1-2 thrombocytopenia (n=9) and grade 3 hepatotoxicity (n=2) were reported.

Conclusions

The significantly increased PFS after inclusion of CCNU in first-line protocols for dogs with HGTCL seems to justify a prospective study with a predetermined CCNU-L-CHOP protocol. Renal toxicity might be a limiting factor using CCNU.
Characterization of stem cell markers in canine B-cell lymphoma

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Introduction
Canine lymphoma represents one of the most common spontaneously occurring tumors in dogs. Cancer stem cells (CSCs) are considered to be responsible for tumor formation, progression, recurrence and metastasis and to play a key role in therapeutic failure rates in several neoplasias. The aim of the present study was to identify CSCs within the canine B-cell lymphoma cell lines CLBL-1 and CLBL-1M. For this purpose, the expression patterns of stem cell marker genes in the two B-cell lymphoma cell lines as well as in primary lymphoma samples were analyzed.

Materials and Methods
Expression of the stem cell marker genes CD34, CD133, CD44, c-KIT, OCT4, KLF4, Nanog, c-MYC, SOX2, DDX5, ITAG6 and MELK in CLBL-1, CLBL-1M cell lines and 14 primary lymphoma samples was analyzed using conventional RT-PCR and real-time PCR. The cell surface markers CD44, CD133, CD34 and CD49f were additionally examined in CLBL-1 and CLBL-1M by flow cytometry.

Results
CLBL-1 and CLBL-1M cell lines had similar expression patterns as primary B-cell lymphoma samples showing expression of CD44, c-MYC, DDX5, ITAG6 and MELK. Flow cytometry analyses showed highly frequent expression of CD44 and weak expression of CD49f in both cell lines. However, there was no CD34 or CD133 positive subpopulation.

Conclusions
Based on the similar expression patterns of selected stem cell markers, CLBL-1 and CLBL-1M can be valuable tools for further research analyzing stem cell markers and the biology of potential tumor stem cells.
CHARACTERISTICS AND OUTCOME OF 17 CASES OF CANINE LYMPHOMA WITH ABERRANT IMMUNOPHENOTYPE

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Introduction
The prognostic significance and response to therapy of aberrant phenotypes in canine lymphoma is unclear. The goal of this study was to determine the clinical presentation and response to chemotherapy of canine aberrant lymphomas.

Materials and Methods
Medical records of the Royal Veterinary College were searched retrospectively for confirmed cases of canine lymphoma. The following antigen patterns were considered aberrant: diminished/absent expression of common leukocytes antigen (i.e CD45), over/under expression of lineage specific antigen, positivity to precursor marker (i.e. CD34), double positivity or double negativity to CD4 and CD8 in T-cell lymphoma, and biphenotype pattern (CD3+CD21+ or CD3+CD79a+). Signalment, clinical presentation, staging, response to therapy, and progression free interval (PFI) were recorded.

Results
17 canine aberrant lymphomas were identified, 15 high-grade and 2 low-grade. Five lymphomas were of B-cell origin (29%), 10 of T-cell origin (59%) and 2 were null (12%). The most common aberration was double negativity to CD4 and CD8 in T-cell lymphoma (29%). The majority of cases presented as stage V (47%) and sub-stage b (76%). Ten cases had unusual localizations of the disease (i.e. lungs). Overall response rate to chemotherapy was 60% with only 2 patients achieving complete response. Responses were generally short-lived (PFI 90 days), 10 patients died or were euthanized due to progressive disease, 5 were lost of follow-up, and only the 2 low-grade lymphomas were still alive.

Conclusions
The majority of aberrant lymphomas of our study presented with negative prognostic factors (stage V, sub-stage b). Further studies are necessary to evaluate whether aberrant lymphomas carry a worse prognosis.
Concurrent T zone and B cell lymphoma in 7 dogs

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Introduction
Molecular diagnostics allow for sensitive detection of canine lymphoma and sub-type discrimination. Treatment decisions are often made based on whether a lymphoma is indolent or aggressive. Using molecular diagnostics we have identified dogs with simultaneous T zone and B cell lymphoma. The presence and persistence of the indolent T zone lymphoma necessitates more critical assessment of treatment response and duration to avoid unnecessary therapy.

Materials and Methods
Retrospective analysis of 7 cases of canine lymphoma where flow cytometry and PARR detected concurrent T zone and B cell lymphoma. Follow-up data including clinical staging, treatment, cytology and sequential molecular diagnostics and outcome were obtained.

Results
In 3 dogs T zone disease was detected prior to the coexisting B cell tumor and in 4 dogs the two tumors were diagnosed simultaneously. Median age at diagnosis of the B cell lymphoma was 11.2 years and 4 of the dogs were Golden retrievers. Median survival time after the diagnosis of B cell lymphoma was 15 months. Four dogs with follow up PARR testing were in molecular remission for the B cell lymphoma while T cell clonality remained detectable. Lack of awareness of the coexistent T zone lymphoma would have resulted in the incorrect clinical assessment of treatment response or remission duration for the B cell lymphoma in 4 of the 7 dogs.

Conclusions
Knowledge of the concurrent presence of T zone and B cell lymphoma in a patient is important to avoid misinterpretation of clinical response to treatment and the necessity for additional therapy.
Genetic heterogeneity of canine DLBCL by Oligonucleotide Array CGH

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Introduction
The role of genomic alterations (GA) at high resolution in Diffuse Large B-cell Lymphoma (DLBCL) has been scarcely investigated in dogs.

Materials and Methods
We analysed 12 newly-diagnosed multicentric DLBCLs using an 180,000 Oligonucleotide aCGH on lymph nodes (LN) samples. aCGH was also repeated on LNs of 3 relapsing dogs and 4 dogs in remission after chemotherapy. All LNs were matched with corresponding skin biopsies.

Results
In pre-treatment DLBCLs, the pattern of GA consisted of 90 different genomic imbalances (mean per tumour, 17), 46 gains and 44 losses. In the LNs of 4 dogs in remission after chemotherapy, 4 new GA were found, whereas 3 new GA were observed in relapsing dogs, compared with the initial sample. Two gains in Chr13 were significantly correlated to stage III-IV of disease (p=0.002). Statistical analysis identified regions of gains and losses significantly associated to time of remission (FDR <0.001).

Conclusions
In pre-treatment DLBCLs, individual variability in the number of GA was found, however 14 recurrent aberrations (frequency >30%) were identified. Losses involving IGK, IGL and IGH were found in all tumors and in 2 dogs in clinical remission. Gains along the length of Chr13 and Chr31 were often found (>41%). In these segments, MYC, LDHB, HSF1, KIT and PDGFRA were annotated. One ex novo GA, involving TCR, was present in dogs in remission after chemotherapy. A reduced number of chromosomal rearrangements was found in relapsed (n=17) DLBCLs when compared with pre-treatment DLBCLs (n=90). Further studies are needed to identify an association with the clinical behaviour of DLBCL.
Expression of Bcl-2 and Ki-67 in 45 feline non-Hodgkin lymphomas

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Introduction
Feline lymphoma is one of the most common hematopoietic tumours in this species. B-cell lymphoma gene 2 (BCL-2) encodes an anti-apoptotic protein, and its expression was previously found in feline lymphoma (mainly T-cell lymphoma). Ki-67 protein is closely associated with cell proliferation and it is used for mytosis identification in histological samples.

Materials and Methods
Forty five Feline non-Hodgkin lymphomas (FnhL) were classified according to the WHO scheme by histopathology and immunohistochemistry (IHC). For IHC we used anti-CD3 antibody (A452; Dako), anti-CD79a antibody (clone HM57; Dako), anti-Pax5 antibody (NCL-L-Pax5, Novocastra), anti-BLA.36 antibody (M0533; Dako), for WHO classification and anti-Bcl-2 antibody (clone 124; Dako) and anti-Ki-67 antibody (clone MIB-1, Dako).

Results
Of all 22 T cell lymphomas 17 (77%) were found to express BCL-2 protein. Of all B cell lymphomas only 8/23 (34,3%) stained positively for BCL-2. In general T-cell lymphomas shown higher Ki-67 indexes (59,1%, more than 10 mytosis per higher power field) than B-cell lymphomas (34,8%, more than 10 mytosis per HPF).

Conclusions
We show in this study the aplicability of BCL-2 and Ki-67 staining in FnhL paraffin-embbeded samples. BCL-2 protein may be of interest for IHC in order to facilitate the distintion between lymphoma and Inflammatory Bowel Disease, to identify a potential therapeutical target and to evaluate its prognostic relevance in this species. Further prospective studies are required to assess BCI-2 prognostic implication on fnHL.
Results of PEG-L-asparaginase (Oncaspar®) incorporation in a modified COP-protocol, or added to prednisolone, for the treatment of high-grade lymphoma in cats

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Introduction
Polyethylene-glycol-L-asparaginase (PEG-ASP) has reduced immunogenicity and longer half-life compared to native L-asparaginase. The objective of this study was to assess Progression-Free-Survival (PFS) in cats with high-grade lymphoma, treated with first-line PEG-ASP-COP or PEG-ASP-prednisolone, designed to reduce the number of vincristine/cyclophosphamide administrations.

Materials and Methods
Records of a veterinary oncology consortium were reviewed (2000-2013). Included were cats, treated minimum one month with ≥ 2 PEG-ASP injections (40 IU/kg i.m. every 2-4 weeks). Continuing PEG-ASP, first injection required tumour reduction. Kaplan-Meier method and log-rank test were used for survival analysis. Retrospective immunophenotyping of gastric and intestinal lymphomas was attempted.

Results
Thirty-two cats were excluded, of which 7 (all high-grade) did not respond to first PEG-ASP. Thirty-eight were enrolled in the study: 32 PEG-ASP-COP and 6 PEG-ASP-prednisolone. CR in PEG-ASP-COP was 81.3%: 5/8 gastric (5B/1Tcell/2 not-phenotyped), 3/4 intestinal (3B/1Tcell), 4/5 other-abdominal, 9/10 nasal, 2/2 multicentric, 3/3 miscellaneous lymphomas. Thirteen cats completed PEG-ASP-COP: median number of 9 vincristine/cyclophosphamide, 13 PEG-ASP doses, median treatment time 296 days. Eleven cats died tumour related, 3 therapy related. Estimated one-/two-year PFS is 67.5/61%. CR in PEG-ASP-prednisolone was 100%: 1 intestinal (not phenotyped), 1 other-abdominal, 2 nasal, 2 multicentric. Three cats completed PEG-ASP-prednisolone: median number of 26 PEG-ASP doses, median treatment time 539 days. Estimated one-/two-year PFS is 62%. PFS for gastric and intestinal lymphoma differed significantly (P=0.044) with a median of 414 and 105 days, respectively.

Conclusions
Treating feline high-grade lymphoma, PEG-ASP can reduce the number of vincristine/cyclophosphamide doses, preserving PFS. Gastric lymphoma may have a better prognosis compared to other alimentary lymphomas.
Canine Melanoma Treated with Autologous Dendritic Cell-Based Vaccines in 10 dogs

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Introduction
The presented research shows the result of an autologous dendritic cell-based cancer treatment in 10 dogs suffering from malignant melanoma in various localizations. The autologous production of dendritic cells and the ability to present autologous antigens to the immune system yields very good clinical results.

Materials and Methods
Through gradient centrifugation and a adherence step, a monocyte culture is extracted from the fresh whole blood of the patient. These monocytes are then cultivated with specific cytokines in order to derive autologous DCs. Finally, the DCs are primed with autologous tumor lysate. The antigen presenting DCs are then harvested, resuspended and injected intradermal in the patient.

Results
10 dogs have been treated with dendritic cell-based cancer vaccines. Median survival time is 785 days after the initial treatment with DC vaccine. Survival rate after 446 days is 66.7% and after 830 days still 40%, remaining constant until the end of the observation period. Due to the fact that 4 of the 10 patients are still alive, the censored result is preliminary and subject to further improvement.

Conclusions
Independent of localization (oral mucosa and different skin localizations), the expected longevity of the patients increased. Together with surgical excision of the cancer cells, the application of DC-based vaccines yields promising results in the treatment of malignant melanoma in dogs, also in rather difficult localizations such as the oral mucosa. Additionally, since no or very slight side effects could be observed, the quality of life of the patients was maintained.
Advantages and limitations of different hybrid imaging methods in veterinary oncology with a special emphasis on staging and restaging canine melanoma cases

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Introduction
Many types of whole body 3D hybrid methods (PET/CT, SPECT/CT) is a daily routine in human centers but not yet in veterinary medicine. Several hybrid imaging techniques using different radiolabelled ligands have a great potential in early and specific primary tumor detection, metastases localization (staging) and follow-up, restaging examinations.

Materials and Methods
Altogether 46 dogs were referred for PET/CT or SPECT/CT examination at our Institute, with known malignant melanoma diseases. In 22 out of 46 dogs two or more hybrid imaging were performed. Dogs were sedated (propofol and diazepam iv, then isoflurane inhalation), injected with different radiopharmaceuticals 40-60 MBq/10 bwkgs (99mTc-MIBI, 99mTc-DMSA(5), 99mTc-colloids, 18FDG, 68Ga-DOTATOC). Whole body native and contrast CTs and nuclear medicine scans were evaluated also separatedly but fusion images were reconstructed, evaluated visually and quantitativelly.

Results
Hybrid imaging detected all the earlier known metastases, and 6 cases we could detect laesions that were not seen in earlier CTs. We found 18FDG and 99mTc-DMSA5 extremelly usefull (with high SUV-values) in melanoma staging and restaging while 68Ga-DOTATOC proved to provide too low SUV values. 99mTc-folate targetting colloid seems to be also a very promissing agent however only 8 melanoma cases were scanned using that radioligand. Sentinel node detection using SPECT/CT before surgery also feasible and makes easier to find the non-enlarged nodes too. All of the applied whole body methods proved to be harmful even in late-stage diseased dogs.

Conclusions
A variety of whole body hybrid imaging methods might be introduced in the near future in staging and restaging our four-legs animal patients.
COARSE FRACTIONATED RADIATION THERAPY FOR THE TREATMENT OF MICROSCOPIC CANINE SOFT TISSUE SARCOMA

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Introduction
Soft tissue sarcoma (STS) is a common canine subcutaneous tumor. Surgery with or without radiation therapy (dependent on surgical margins) is the current standard of care in dogs. Typical protocols for treating incompletely excised STS involve curative intent radiation with total dose in excess of 50 Gy. The purpose of this study was to evaluate progression free survival (PFS), time to local failure (TLF) and overall survival (OS) for canine STS treated with an hypofractionated radiation therapy (RT) protocol.

Materials and Methods
Histologically confirmed microscopically incomplete or closely excised STS confined to the primary site were treated with once weekly fractions of radiation (6 - 8 Gy/fraction) to a total dose of 24-36 Gy.

Results
Forty-eight dogs were included. Histologic grade was available for 44 dogs (13 grade 1, 22 grade 2 and 9 grade 3). Total radiation dose ranged from 24 to 36 Gy. Eleven dogs (23%) developed local recurrence. Twelve dogs (25%) developed metastasis. The median PFS probability was calculated to be 698 days (with 1, 2 and 3 years PFS of 63%, 44% and 23%), the median TLF was not reached (with 1, 2 and 3 years TLF of 81%, 73% and 73%) and the median OS was not reached (with 1, 2 and 3 years OS of 81%, 75% and 61%). Only histologic grade was prognostic for PFS and OS.

Conclusions
The hypofractionated RT protocol employed achieved long-term local tumor control in the majority of dogs. It is, therefore, reasonable to prescribe in older patients or when financial limitations exist.
CANINE MENINGIOMA: COMPARISON OF PALLIATIVE THERAPY, SURGERY AND STEREOTACTIC RADIOSURGERY

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Introduction
Meningiomas represents about half of primary intracranial tumours in dogs. There are limited comparative studies regarding the various treatment modalities. Aim of this study was to compare palliative therapy, surgery and stereotactic radiosurgery in canine meningioma.

Materials and Methods
Data were collected retrospectively from 198 dogs referred to one institution over a 15-year period with histopathologically confirmed or MRI consistent with meningioma. Dogs were grouped by anatomical site (supratentorial - E, infratentorial - T, spinal - S) and by therapeutic option (palliation - P, surgery - S, radiosurgery - R). Surgery goal was total tumour resection. LINAC based VMAT radiosurgery was performed in 1 - 5 fractions. Serial clinical and MRI examinations were conducted. Signalment, clinical signs, neuroanatomic tumour location, relapse specifics, adverse events, best response and overall survival (OS) time were evaluated. OS estimates were calculated using Kaplan-Meier method and the differences between groups compared using logrank analysis.

Results
91 dogs (51 E, 33 T, 7 S) had been palliated, 69 dogs (33 E, 31 T, 5 S) had been treated with stereotactic radiosurgery, 38 dogs (32 E, 1 T, 5 S) with surgery. OS in PE was 190 days, in PT 38 days, in PS 89 days, in RE 781 days, in RT 654 days, in RS 813 days, in SE 567 days, in ST 3 days, in SS 210 days. Variables predictive of OS are localisation and therapy option.

Conclusions
Dogs suffering from meningioma undergoing stereotactic radiosurgery had superior outcome to those treated with surgery or palliation.
Colloid gold nanoparticles conjunct with doxorubicin for feline injection-site sarcomas treatment - new preclinical oncological studies

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Introduction
Feline injection-site sarcomas (ISS) treatment is a challenge for veterinary practitioners. Standard treatment includes: surgery, radiotherapy and chemotherapy. Doxorubicin is a first choice therapeutic agent, however, its effectiveness is debatable. The main aim of the study was to assess the effectiveness of colloid gold nanoparticles conjunct with doxorubicin (DoxAu) using novel chick embryo chorioallantoic membrane (CAM) model.

Materials and Methods
DoxAu was prepared using non-covalently attached doxorubicin (Dox) and glutathione passivated (4nm) gold nanoparticles (Au). Feline fibrosarcoma cell lines (FSS1WAW, FFS1, FFS3, FF5) were cultivated in DMEM enriched with glucose under standard condition. Performed in vitro tests: MTT assay, test with Annexin V and verapamil CAM assay: on the 6th day of chick embryo incubation a sterile silicon ring was put into CAM of each egg. Then 5x10⁶ FFS1 and FFS3 cells were inoculated exactly into silicon rings. After 10 days tumors growth was measured. Tumor bearing chick embryos were divided into 4 groups and Dox, DoxAu, Au and saline were injected intratumoral (in DoxAu IC50 doses obtained in MTT assay). After 72 hours tumors were measured one more time. Statistical analyses were performed using Graph Pad Prism 5.0.

Results
DoxAu was more cytotoxic than free Dox for fibrosarcoma cell lines with high glycoprotein P activity (FSS1WAW, FFS1, FF3). DoxAu significantly inhibits tumors growth after single intratumoral injection.

Conclusions
DoxAu is a potent new therapeutic agent for ISS treatment. CAM model is a good model to assess the effectiveness of anticancer drugs.

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Coarse Fractionated Radiotherapy for Canine Soft Tissue Sarcoma: A Retrospective Study of 97 Cases Treated with 5x6Gy

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Introduction
Wide surgical resection or a marginal/incomplete resection followed by full-course radiation therapy (RT) is the current standard of care for canine STS. Shorter, coarse fractionated RT protocols are often used for inoperable cases, elderly, or co-morbid patients, but also due to financial or logistical restraints of treatment machine availability. The herein presented results of a retrospective, bi-institutional study describe efficacy and toxicity of a 5x6Gy protocol on macroscopic and microscopic canine STS in terms of tumor progression (PFI) and overall survival (OS), identifying prognostic factors on outcome.

Materials and Methods
Dogs with microscopic or macroscopic STS irradiated with 5x6Gy were included. RT (electron field or 3D-conformal 6MV photons) was administered with palliative intent. PFI and OS were compared with respect to different tumor and patient characteristics by the Kaplan-Meier-method and multiple Cox-regression analysis.

Results
97 dogs were included: 18 had microscopic disease and 79 had macroscopic disease. All dogs received the same RT protocol; part of the macroscopic group received post-radiation metronomic chemotherapy. Median PFI for microscopic disease was not reached and longer than for macroscopic disease with 378 days (95%CI:322-434) (p=0.04). There was a trend for longer OS (all deaths) for microscopic vs macroscopic disease with 645 (95%CI:168-1122) and 380 days (95%CI:285-507), respectively (p=0.07). Toxicity was low in both groups. Neither for PFI nor for OS significance was found in outcome of the group treated with additional metronomic chemotherapy (n=32) or without (n=47).

Conclusions
The 5x6Gy RT protocol provided long PFI in the microscopic disease setting, and reasonably prolonged stabilization in the macroscopic setting.
Generation and characterisation of an EGFP-HMGA2 in vitro model for canine prostate cancer

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Introduction
The architectural transcription factor HMGA2 is abundantly expressed during embryonic development but mostly undetectable in differentiated tissues. Several malignant neoplasms including prostate cancer show high re-expression of HMGA2 correlating with malignancy and poor prognosis. HMGA2 is negatively regulated by let-7 miRNAs and the HMGA2-let-7 balance plays a major role in tumour aetiology. To analyse the role of HMGA2 in prostate cancer, a stable, highly reproducible in vitro model system is precondition. Therefore, we established a canine EGFP-HMGA2 prostate cancer cell line stably overexpressing HMGA2 linked to EGFP (Enhanced Green Fluorescent Protein). To exclude EGFP-induced effects, an EGFP-expressing reference cell line was created.

Materials and Methods
Both recombinant cell lines were characterised by fluorescence microscopy, flow cytometry and immunocytochemistry. The proliferative effect of ectopically overexpressed HMGA2 was determined via proliferation assays and the chromosome consistency of the derived cell lines was analysed comparatively versus native cells. The impact of overexpressed HMGA2 on let-7a was analysed via real-time PCR.

Results
Fluorescence microscopy and immunocytochemistry detected successful EGFP-HMGA2 fusion protein expression. The HMGA2 overexpression in EGFP-HMGA2 cells was confirmed by real-time PCR. Significantly higher let-7a expression levels were detected in both fluorescent cell lines. The HMGA2-EGFP cell line showed increased proliferation compared to EGFP- and native cells. Cytogenetic analyses of both fluorescent cell lines resulted in a comparable hyperdiploid karyotype as described for the native cell line.

Conclusions
The new fluorescent EGFP-HMGA2 cell line is a stable tool enabling further in vitro and in vivo analyses of HMGA2-mediated effects potentially being involved in the pathogenesis of prostate cancer.
VACCINATION WITH VIRUS-LIKE PARTICLES INDUCES LONG LASTING PROTECTION FROM EXPERIMENTALLY INDUCED SARKOID-LIKE TUMOURS IN HORSES

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Introduction
We have already demonstrated that vaccination with empty BPV1 capsids termed virus-like particles (VLP) protects horses from experimental infection with BPV1 virion and associated tumour formation. Long term monitoring of antibody titres in experimental horses and data from other species suggest that this protection is long lasting.

Materials and Methods
Seven horses, vaccinated in 2007/2008 with different doses of BPV1 L1 VLP and 3 unvaccinated control horses were challenged by intra-dermal inoculation with cow wart derived BPV1 virion (5x10^7 BPV-1 virions per wheal, 10 wheals per horse). Inoculation sites were monitored for 10 weeks. Blood for serum antibody titre determination by pseudovirion neutralisation assay was taken on the day of challenge and after 6 months.

Results
Six of 7 vaccinated horses had measurable serum antibody titres (1:50 – 1:400). These titres were boosted by inoculation (about one step of dilution). Two of 3 unvaccinated controls remained sero-negative. One control showed sero-conversion. All controls developed tumours at all 10 inoculation sites. Tumours appeared approximately 2 weeks after inoculation and reached maximum sizes of up to 8 mm. Regression was complete by 8 weeks after their first appearance in all horses. All vaccinated horses remained completely free from tumours.

Conclusions
BPV-1 L1 VLP vaccination proves to be fully effective in protecting horses from experimental infection and tumour formation 5 years post immunisation. The protection was complete even in horses with low or unmeasurable antibody titers. This is another step towards establishing a vaccination against equine sarcoiids.
Targeting multiple oncogenes simultaneously improves response to therapy and circumvents acquired resistance to single target tyrosine kinase inhibitors

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Introduction
Epidermal growth factor receptors 1 and 2 (EGFR, ErbB2), and cytosolic Src (c Src) are tyrosine kinases that stimulate cell survival, proliferation and migration and if dysregulated can act as oncogenes. Several tyrosine kinase inhibitors (TKIs) have recently gained approval, but long term treatment with TKIs against a single target often lead to acquired resistance. The aim of this study was to investigate the effect of targeting multiple signalling pathways simultaneously in naïve and TKI-resistant cell lines.

Materials and Methods
The ErbB2 and c-Src genes were sequenced and small interfering RNAs (siRNAs) targeting them were developed. Effective siRNAs as well as ErbB2, Egfr and c-Src TKIs (AG828, gefitinib, GW583340 and saracatinib) were evaluated in canine and feline carcinoma cell lines. The effects of combined RNAi targeting of Egfr, ErbB2 and c-Src were assessed.

Results
Inhibition of c-Src and Egfr inhibited cell proliferation, migration and colony formation in feline and canine carcinoma cell lines while ErbB2 targeting exhibited no effect. Chronic drug exposure with gefitinib and saracatinib lead to resistance which was not associated with ATP binding site mutations. RNA interference (RNAi) strategies targeting Egfr plus ErbB2 and c Src plus either Egfr or ErbB2 showed a synergistic inhibition of cell proliferation. When targeting multiple areas of an oncogene or multiple oncogenes simultaneously significantly lower concentrations of siRNAs were required. Cells rendered resistant by chronic drug exposure were still sensitive to RNAi.

Conclusions
Targeting multiple pathways simultaneously improves growth inhibition, reduces the siRNA concentrations required and are still effective in cells rendered resistant to TKIs.
Poster presentations

The ESVON Poster Award aims to encourage members in the earlier years of their career in veterinary oncology to pursue a period of scientific investigation in the field of veterinary oncology.

The aim of this award is to recognize outstanding contributions to the knowledge related to pathogenesis, diagnosis, therapy, prevention, or control of animal tumour-diseases. It is given once yearly at the ESVONC Annual General Congress to a veterinarian based upon his/her written abstract and poster presentation of scientific data at the congress. In addition to a Certificate this award covers the travel, hotel and registration costs with a maximum of 1000,- Euro for the author to attend a congress of his/her own choice in Europe.
Adrenal tumours with vascular invasion: stereotactic hypofractionated volume modulated arc radiotherapy (VMAT) in 10 dogs

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Introduction
Surgery is the standard treatment of adrenal tumours, but it is full of potential difficulties, particularly in cases of intracaval extension. Aim of this study was to evaluate the technical feasibility and therapeutic efficacy of adrenal tumours ablation using hypofractionated volumetric modulated arc stereotactic radiotherapy (VMAT) in dogs with vascular invasion.

Materials and Methods
10 dogs suffering from adrenal tumours with vascular invasion were treated using a LINAC with micromultileaf collimator. The VMAT plans were computed using a MonteCarlo statistic algorithm and the Monaco 3.0 treatment planning system. Dose constraints have been derived from AAPM TG 101. The plan has been evaluated for quality assessment with DosimetryCheck. The prescription dose was 33-39 Gy in 3 fractions. The follow up have provided biochemical exams and CT/MRI.

Results
According to RECIST criteria, 7 partial responses and 3 stable diseases were obtained, but all patients showed a better performance status. Ascites disappeared immediately after the treatment completion. At 1 year all patients are alive. In 3/4 dogs with an elevated cortisol level, a value normalisation was obtained. According to VRTOG criteria, a grade I pancreatitis and a focal renal cortical atrophy were observed.

Conclusions
Results of this study are better than the median survival time reported in literature. Stereotactic hypofractionated VMAT may be an option in cancer treatment and control of endocrine clinical signs, with a low morbidity and mortality rates. Further studies of dose escalation are suitable.
Rabbits thymoma: optimisation of image-guided dynamic IMRT set up

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Introduction
Radiotherapy is a treatment option for rabbit thymoma but toxicity is frequent. The aim of this study was to define an optimised plan set up to reduce the incidence of complications. The hypothesis was that image-guided dynamic IMRT would allow to deliver higher doses to the target with better sparing of organs at risk in comparison to 3D conformal radiotherapy.

Materials and Methods
Five rabbits suffering from thymoma were treated with LINAC-based hypofractionated dynamic IMRT. Dose prescription was 40 Gy in 6 fractions. Throughout treatment, 3 subsequent CT/MRI scans were performed; positioning was verified with cone beam CT. Treatment plans were computed with MonteCarlo statistic algorithm and Monaco 3.0 planning system, verified with DosimetryCheck system. Parameters evaluated were: number and width of arches, control points, modulation degree, margin to target and organs at risk. The goals were: obtained V95% >95%, target EUD >95%, V107%

Results
Prescription goal were obtained in all five cases with 1 arc of 360°, 137 control points, 2.5 modulation degree, 2 mm margin to target and 3 mm margin to organs at risk. In all patients tumour disappeared and at 1 year no complications were observed.

Conclusions
Image-guided dynamic IMRT with suitable parameters is feasible in rabbits. No morbidity or mortality events were reported in animals treated. Treatment schedule is effective in disease control and usable in clinical setting.
EFFICACY OF EXTERNAL BEAM RADIATION COMPARING TO GADOLINIUM NEUTRON CAPTURE THERAPY RADIATION FOR DOGS WITH III STAGE MALIGNANT ORAL MELANOMA

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Introduction
Neutron capture therapy (NCT) is an experimental treatment with low energy thermal neutrons used for human patients with head and neck cancer. Gadolinium neutron capture therapy (GdNCT) was compared to hypofractionated radiation therapy in dogs with oral malignant melanoma (OMM) for local tumor control and survival times.

Materials and Methods
Dogs with III stage OMM were divided into two groups. Hypofractionated radiation therapy was performed in 33 dogs (group 1) in a total dose of 50-60 Gy once a week in 7.5 Gy fractions. Group 2 of 34 dogs was treated with GdNCT. Neutron capture therapy was reformed at the IRT MEPHI reactor with thermal neutron flux 1.1×10^9 n/cm^2/s. Gadolinium-containing drug Gd-DTPA was administered intratumoral immediately prior to irradiation. In dogs with confirmed regional lymph node metastasis lymphadenectomy was also performed. Response rates (PR – partial remission, CR – complete remission) and overall survival time were recorded in each group.

Results
In the external beam radiation therapy group 45.5% dogs had PR and 30.3% CR. In the GdNCT group PR was detected in 50.0% of dogs and CR in 47.1%. Although there were no statistical significant differences in local tumor responses in the two groups, the overall survival differed significant – 132.7 days in the radiation therapy group comparing to 204.2 days in the GdNCT group (p=0.049).

Conclusions
GdNCT is effective in local disease control of canine oral melanoma III stage and in our study significant enhanced overall survival times comparing to external beam radiation.
Generation and Validation of a Gene Expression Signature for Metastasis in Canine Soft Tissue Sarcomas

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Introduction
Histopathologic grade is currently used to determine the metastatic potential of canine soft tissue sarcomas (STS). Grading schemes for STS are subjective and variable. At best, high-grade STS have a 30-40% metastatic rate, resulting in many dogs potentially receiving chemotherapy who will not benefit and may be harmed. Our hypothesis is that utilization of a gene expression signature (GES) would be able to predict future development of metastasis with 70% accuracy.

Materials and Methods
Canine Genome 2.0 microarrays (Affymetrix) were run on 12 metastatic STS and 24 non-metastatic STS (with at least 1 year follow-up). There were 804 probes identified as differentially expressed between these two groups at the 2-fold level. After deletion of probes without known genes associated with them, and averaging across probesets for individual genes, 322 genes remained. Random Forest analysis was used to determine the relative importance of each gene in separating the two data sets, as well as the classification error produced by each set of genes included. The final GES was validated with qPCR in a second set of 31 STS evenly divided between metastatic and non-metastatic tumors.

Results
Between 4 and 16 of the most important genes gave the highest correct classification of the STS. qPCR of these 16 genes has been performed in 31 additional STS, and the predictive value of this GES is currently being calculated, along with the minimal number of genes necessary to accurately classify a STS as metastatic or non-metastatic.

Conclusions
The final step will be prospective evaluation of the GES.
The long-acting COX-2 inhibitor mavacoxib (Trocoxil™) has anti-proliferative and pro-apoptotic effects on canine cancer cell lines and cancer stem cells in vitro

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Introduction
The NSAID mavacoxib (Trocoxil™) is a selective COX-2 inhibitor used for the management of inflammatory disease in dogs. It has a long plasma half-life, requiring less frequent dosing and supporting increased owner compliance in treating their dogs. COX-2 overexpression is associated with tumorigenesis in dogs. In this study we compared the in vitro activity of a short-acting non-selective COX inhibitor (carprofen) with mavacoxib, on cancer cell and cancer stem cell survival.

Materials and Methods
A panel of canine cell lines was tested including KTOSA5 (osteosarcoma), CSKOS (osteosarcoma), D17 (osteosarcoma), J3T (glioma), C2-S (hemangiosarcoma), SB (hemangiosarcoma), 3132 (lymphoma), and CPEK (normal epidermal keratinocyte). Putative cancer stem cells were isolated from KTOSA5 cells by cell sorting for CD34. To examine sensitivity to these NSAIDs, we assayed for cell viability and colony forming ability. The effect of NSAIDs on apoptosis was determined by annexin V-FITC staining.

Results
Using a panel of canine cancer cell lines we demonstrate that both carprofen and mavacoxib has a direct cell killing effect on cancer cells, and increases apoptosis in cancer cells in a manner that may be independent of caspase activity. Furthermore, mavacoxib, but not carprofen, is cytotoxic to cancer stem cells derived from osteosarcoma cell lines.

Conclusions
Both NSAIDs can inhibit cancer cell proliferation and induce apoptosis in vitro. Importantly, cancer stem cells derived from an osteosarcoma cell line are sensitive to the cytotoxic effect of mavacoxib. Our results suggest that mavacoxib has anti-tumour effects and that this in vitro anti-cancer activity warrants further study.
Long term tolerance in dogs with selected neoplasia undergoing continuous low dose chemotherapy: results in 75 dogs

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Introduction
Low dose continuous (metronomic) chemotherapy (MC) is a now well-recognized therapeutic option in dogs with various tumors, acting by inhibition of angiogenesis and immunologic modulation. It has recently been demonstrated that a dosage of 15 mg/m² of cyclophosphamide is required to achieve inhibition of angiogenesis. However, long term safety of continuous treatment with this continuous dosage of cyclophosphamide remains undetermined.

Materials and Methods
Dogs presented at the Oncovet center, in which MC was proposed as part of the treatment of well-identified and staged tumors were retrospectively reviewed. Follow up was assessed, with registration of side effects, associated treatment and outcome.

Results
A total of 75 cases, receiving an initially-planed daily dosage ranging from 15 to 25 mg/m² were identified. This includes 30 dogs with various carcinoma, 10 dogs with hemangiosarcoma, 10 dogs with oral melanoma, 9 dogs with osteosarcoma and 16 dogs with soft tissue sarcoma. Treatment with MC was used as part of a multimodal therapy was usually well tolerated. Sterile hemorrhagic cystitis (SHC) was the main side effect and was observed in 19% of dogs. Two-thirds of dogs with SHC were female.

Conclusions
The use of a continuous treatment with a 15-to-25 mg/m² dosage of cyclophosphamide is associated with a relatively high incidence of SHC, particularly in female dogs. Frequent qualitative monitoring of urine is recommended in dogs undergoing MC with cyclophosphamide.
Chromosome preparation from canine whole blood and tumor cells

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Introduction
Tumour cytogenetics is focused on detection of specific tumour-associated chromosome aberrations. To do so, a sufficient number of qualitatively good metaphases are prerequisites. Chromosome preparation from canine cells is not as simple as in human beings, as available human standard protocols cannot be transferred directly on canine cells. Thus, the aim herein was to establish reliable standard protocols for preparation of canine cells for routine chromosome analysis.

Materials and Methods
Blood samples from healthy dogs (n=10) and two different cultures of mammary tumour cells (n=10 each, designated as T120 and T121) were analysed. The blood cells were cultured in different media (Chromosome Medium B®, Chromosome Medium P® and LymphoGrow®) and the tumour cells in Medium 199® at 37°C with 5% CO2/air. To arrest the cells in metaphase, 62.5µL of 0.05M colcemid/mL were used for blood cells during 30 min and 14µL/mL for tumour cells during 2h. Hereafter, the cells were swollen using hypotonic solutions (tumour cells – 1:6 medium:ddH2O; blood - 0.05M KCL) and fixed with methanol:glacial acetic acid (3:1). The fixed cell suspensions were dropped onto ice-cold slides and dried prior to G-banding.

Results
For blood sample preparation, specific conical tubes (TPP AG) and Chromosome Medium P® showed the best outcome to gain numerous mitotic cells. For tumour cell cultures, variation in the handling steps resulted in qualitatively good metaphases, revealing a complete canine chromosome set for T120A while T121 showed varying chromosomes numbers (67-77).

Conclusions
Modified human protocols allow generation of metaphases in sufficient number and quality, enabling us to perform routine analyses.
Is prognosis better than we thought?! - A retrospective analysis of 40 canine gingival squamous cell carcinomas receiving radical surgery

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Introduction
40 dogs with surgically treated gingival squamous cell carcinomas (SCC) were analysed retrospectively regarding breed, age, tumor localisation, postsurgical complications, and survival times (ST). Prognostic factors were evaluated.

Materials and Methods
Patients were staged using CT scans of the head and lungs and palpation/FNA of tributary lymph nodes. All dogs were treated with jaw resections. Dogs with metastatic disease received adjuvant Carboplatin chemotherapy.

Results
Medium to large breeds and male dogs were overrepresented. Median ST of all patients was 44.8 months. There was no significant difference in median ST for maxillary (n=15) and mandibular tumors (n=25) (p=0.985). On multivariate analysis only tumor stage was significantly associated with ST (p=0.0047). 38/40 (95%) of the patients were N0 upon presentation and reached a median ST of 44 months. Two patients with N1 survived 18 and 70 months following jaw resection and Carboplatin chemotherapy. Of the 5 patients with unclean resection margins (ST 6-146 months) only one dog experienced tumor recurrence after 5 months. In 2 dogs postsurgical suture dehiscence occurred. Functional outcome was good to excellent in all dogs.

Conclusions
Prognosis in canine gingival SCC depends on tumor stage. Complete excision carries a good prognosis and in the majority of cases a cure can be expected. Patients with metastasis to local lymph nodes can have acceptable STs. The allegedly more malignant behaviour of caudally located gingival SCCs could not be confirmed. Jaw resections bear a low rate of complications.
Treatment of canine mast cell tumors with radiotherapy, toceranib, chlorambucil and prednisone

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Introduction
Canine mast cell tumors (MCT) of high grade, non-resectable, with low margins or recurrent after surgery require adjuvant therapies. Aim of this study was to evaluate the toxicity and the efficacy of radiotherapy (RT) with concomitant and subsequent toceranib, chlorambucil and prednisone.

Materials and Methods
Fourteen dogs were treated with curative RT for MCT (three G2SI of lips, one G3SIII of hard palate, one G2SIII of digit), with adjuvant RT for MCT recurrent after surgery (two G3SIII of shoulder muscles) or resected with low margins (three G2 of interscapular muscles, two G2 of stifle skin, two G2 of penis skin). The dose to the tumor bed and local lymph nodes was 4 x 800cGy fractions at 7-day intervals. During the treatment, toceranib (2.5 mg/kg/48h), chlorambucil (0.1 mg/kg/24h) and prednisone (0.5 mg/kg/24h) was administered, continuing toceranib for 6 months, chlorambucil and prednisone for 2 months. Toxicity, response, and survival were evaluated along 2.5 years.

Results
All the dogs achieved a complete remission. All three dogs with MCT G3 experienced local recurrence and metastasis, with progression free interval (PFI) of 8 months and median survival time (MST) of 13 months. Median PFI and MST was not reached in dogs with MCT G2. Six of 14 dogs have requested temporary suspension of chemotherapy. Five and three dogs experienced, respectively, grade II acute and grade I late radiation morbidity.

Conclusions
The protocol is useful in the treatment of canine MCT with slight toxicity, however an extension of the study is advisable.
Molecular characterization of canine mammary tumours: the role of miRs and mRNAs as biomarkers in the metastatic transition

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Introduction
Differential expression of microRNAs (miRs) has been associated with tumour suppressive and oncogenic patterns in Canine Mammary Tumours (CMTs). However, their clinical value as cancer biomarkers has not been completely proved. The analysis of the expression of 10 miR's (miR-16, miR-21, miR-24, miR-29b, miR 124, miR-155, miR-199b, miR-200c, miR-203, let-7a) and mRNAs in CMTs may contribute for the understanding of the molecular basis of CMT tumorogenesis and could prompt the discovery of CMT valuable biomarkers.

Materials and Methods
Total RNA was extracted from 4 biopsies of paired normal/tumour tissue samples preserved in RNAlater. High quality total RNA (RIN values between 7.6-9.4) was used for cDNA synthesis. miRs were quantified using LNA Real-Time PCR (Exiqon, Denmark) assay and mRNAs were quantified using RT-PCR with Evagreen (Solis BioDyne, Estonia).

Results
Our observations revealed an increased expression of DICER1 in non-metastatic CMT samples and underexpression in the metastatic CMT. From the 10 miR's tested we also observed decreased expression of miR-16, miR-199b and increased expression of miR-21, characteristic of CMTs without metastasis. The metastatic cancer analysed had a distinctive increase in the expression of miR-155.

Conclusions
Our results seem to support the importance of DICER1 and miR expression in the metastatic transition in CMTs. Correspondingly, in human breast cancer, loss of DICER1 expression is correlated with cancer progression and recurrence. Furthermore, with the acquisition of metastatic ability, the miR expression profile changes can be molecular markers of malignancy in CMTs. Due to our low number of samples, we continue to collect more CMTs to complement our result.
Efficacy and side effects of radiation therapy in comparison with radiation therapy and temozolomide in the treatment of canine malignant melanoma

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Introduction
In dogs, malignant melanoma (MM) is notorious for its propensity to metastasize and for its poor response to current therapeutic regimens. Better tolerated, less toxic, and more efficacious treatments are needed.

Materials and Methods
Dogs with newly diagnosed or recurrent, histologically confirmed MM of any clinical stage were prospectively enrolled to investigate objective response, time to progression (TTP), and overall survival as well as the safety profile of radiation therapy (RT) in comparison with RT and temozolomide. The RT protocol consisted of 5 fractions of 6 Gy to a total dose of 30 Gy over 2,5 weeks. Dogs whose owners wished to pursue chemotherapy received adjuvant oral temozolomide (60 mg/m²/die for 5 days). Treatment cycles were repeated every 28 days for 4 cycles.

Results
Fifteen dogs were treated with RT (Group 1) and 12 dogs were irradiated and subsequently treated with temozolomide (Group 2). Response rate was 73.3% (3 complete remissions [CR], 8 partial remissions [PR]) in Group 1, and 75% (2 CR, 7 PR) in Group 2. Median TTP was not significantly different among groups. When considering survival time, dogs in Group 2 lived significantly longer than dogs in Group 1 (203 days versus 165 days; p = 0.044). Toxicity was similar between groups.

Conclusions
Conventionally fractionated RT followed by 4 cycles of temozolomide has a good safety profile and increases survival time in dogs with MM. These results warrant future randomized trials to further explore the role of adjuvant temozolomide, and support the general concept of combining radiation therapy with radioenhancing chemotherapeutic agents.
Treatment of advanced canine MCT with Palladia® (toceranib phosphate)

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Introduction
This study reports efficacy of toceranib in advanced mast cell tumours (MCT).

Materials and Methods
This open label multicenter study recruited dogs with recurrent, non-resectable grade II or III MCTs with minimum estimated life expectancy of at least 12 weeks, appropriate clinical staging, and no significant co-morbidities. RECIST tumour response, adverse effects and drug dosages were recorded.

Results
93 dogs (51 female, 42 male) were included. Mean age and body weight were 8.9 years (2.6-15.8) and 25.8 kg, respectively. Breeds included Labradors (15%), crossbreeds (13%) and Boxers (10%). Previous treatments included surgery (80%), chemotherapy (46%) chemotherapy and radiotherapy (3%). Dogs had cutaneous (51%) or subcutaneous (60%) tumours (grade II, 69.6%; grade III, 30.4%) located on the head/neck (30.1%), legs (37.6%) and trunk (20.4%). 70% of dogs had metastasis (lymph node 58%; distant 12% (splenic 6%)). Objective response was obtained in 48.4%, and biological response in 82.8%.

Mean dose was 3.17 mg/kg (2.5-3.75), every other day. Dose alterations were made in 52 dogs, and drug “holidays” in 45 dogs. 9 non-serious and 18 serious adverse events were reported. 13 dogs were euthanized for reasons possibly related to drug related side effects and/or disease progression.

Conclusions
Response rates were encouraging given the high percentage of dogs with metastatic disease. While the recommended initial dose level is 3.25 mg/kg it is now common practice amongst oncologists to use lower start doses and lower dose ranges of toceranib without having a negative effect on efficacy (Bernabe et al, 2013; London et al 2012).
ERBB2 oncogene DNA copy number, mRNA and protein expression studies in cat mammary tumours

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Introduction
ERBB2 is a key proto-oncogene involved in human breast cancer onset, aggressiveness, recurrence, mortality and tumour chemoresistance. Our purpose was to evaluate the cat ERBB2 gene expression profile in neoplastic and non-neoplastic mammary lesion samples in order to contribute to the study of the clinical relevance of the ERBB2 gene in cat mammary tumours.

Materials and Methods
ERBB2 DNA copy number (n=10) and mRNA (n=23) status was assessed by qRT-PCR. Protein expression was determined by immunohistochemistry (IHC) using two antibodies (n=33) tested by western blotting. Results were correlated with the clinicopathological traits of cat mammary lesions (CMLs).

Results
CMLs evidenced a lower percentage of erbB-2 positive tissues than normal samples (CB11/internal domain: 51,51% vs 91,7%; CBE356/external domain: 33,33% vs 66,7%). The percentage of positive CMLs was lower for CBE356/external than for CB11/internal labelling. These two anti-human erbB-2 antibodies were validated for cat species by western blotting onto cat mammary protein extracts. No ERBB2 gene amplification was observed.

Conclusions
Consistently, the IHC patterns of the erbB-2 protein revealed a disparity in the membrane labelling between the two antibodies. We only found a positive correlation between the ERBB2 RNA levels and the protein expression labelling of the intracellular region. These results suggest that the ERBB2 gene is post-transcriptionally regulated and that proteins with truncations are present in cat mammary neoplastic lesions. Additionally, in our subset of cat mammary tumours, we found an association between low ERBB2 RNA and erbB-2 protein expression levels, without gene amplification, and poor prognosis. The first two authors contributed equally to this work.
High COX-2 expression is associated with increased angiogenesis, proliferation and tumoural inflammatory infiltrate in canine mammary tumours

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Introduction

COX-2 expression has been shown to affect human mammary tumorigenesis via a number of distinct mechanisms: promoting tumour maintenance and development, inducing angiogenesis and cell proliferation, encouraging metastatic spread and tumour associated inflammation. In order to better understand this subject in canine mammary tumours (CMT) the role of COX-2 was investigated by searching the potential relationship with angiogenesis, proliferation and inflammatory infiltrate in a large series of cases.

Materials and Methods

One hundred and eleven CMT (90 malignant and 21 benign) were submitted to immunohistochemical staining to detect the immunoexpression of the following marker: COX-2, Ki-67, CD31, VEGF, CD3 and MAC387.

Results

Considering the group of malignant tumours (n=90), high COX-2 immunoexpression revealed a statistically significant association with CD31 (P = 0.021); VEGF (P (r = 0.457; P (r = 0.25; P = 0.026); CD3+ T-lymphocytes (r = 0.287; P = 0.06) and MAC387 (r = 0.285; P = 0.021). In benign tumours (n=21), COX-2 showed no association and/or correlation with the molecular markers considered in the study.

Conclusions

Present study demonstrated a link between high COX-2 immunoexpression and increased angiogenesis, proliferation and tumoural T-lymphocyte and Macrophage infiltration, parameters already described as being related with the promotion of tumoural aggressiveness in CMT. These findings reinforce the usefulness of using selective COX-2 inhibitors as a valuable therapeutic tool in malignant CMT treatment.
PCR for Antigen Receptor Gene Rearrangement as an Adjunct Tool in the Characterisation of IBD

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Introduction
In the pathogenesis of canine Inflammatory Bowel Disease (IBD) an aberrant chronic antigenic stimulation plays a crucial role in the clonal expansion of certain lymphocyte subsets. This study is aimed at investigating the immunoglobulin (Ig) and T-cell receptor (TCR) gene rearrangement in dogs affected by IBD to identify clonal expanded lymphocytes.

Materials and Methods
From 36 dogs suffering from IBD and six healthy controls, intestinal biopsies of the duodenum and colon were taken and examined by histopathology and immuno-histochemistry. In total, 77 snap-frozen bioptic samples were retrieved and the genomic DNA (gDNA) was purified following standard protocols. To assess clonality, gDNA samples were subjected to triplicate PCR using primers targeting the variable regions of B- and T-cell receptors (PARR). Size-separation of the Ig and TCR gene rearrangements was performed by high-resolution capillary electrophoresis.

Results
All cases showed an Ig polyclonal pattern indicating a non-tumorigenic status. Four patients out of 36 (11.1%) exhibited reduced diversity of the TCR repertoire by displaying oligoclonal or monoclonal peaks in the electropherogram. T-cell clonality of these patients was verified by sequencing the rearranged TCR segments.

Conclusions
To date, PARR is the only test, able to confirm clonality in a population of canine lymphoid cells. Four of the IBD patients were identified having an intestinal clonal T-cell population. This leads to the recommendation of using PARR as an adjunct diagnostic tool in early detection of a borderline state from severe chronic inflammation to clonal expansion of certain lymphocyte subsets in the pathogenesis of canine IBD.
Evaluation of COX-2 and MDR1 expressions in canine mammary gland tumours

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Introduction
Cyclo-oxigenase-2 (COX-2) and multidrug resistance protein 1 (MDR1 or Pgp) are considered to have prognostic value in canine neoplasms, like mammary gland tumours. Although, debating research findings has been issued about the practical usage of these markers.

Materials and Methods
Mammary gland neoplasms (n=33) were analysed. After routine histopathological evaluation, we performed immunohistochemistry to detect COX-2 (monoclonal rat IgG1 368-604), and MDR1 (monoclonal mouse Ig G, C494, Signet Laboratories). Tumour bearing dogs had been treated either by chemotherapy, and/or NSAIDs. Data of the efficacy of therapy was noted. Four groups were set up. 1. low co-expression (<40% MDR1 and COX-2, n=8); 2. high co-expression (>40% MDR1 and COX-2, n=7); 3. high MDR1 expression (MDR1>40%, COX-2<40%, n=9); 4. high COX-2 expression (MDR1<40%, COX-2>40%, n=9).

Results
Median overall survival times (days) were different in groups. 1. 152 ±478; 2. 90.5 (±145); 3. 427 (±561) 4. 19 (±163), respectively. Significant differences were found between high co-expression and high MDR1 expression (p=0.0278); between high MDR1, and high COX-2 expression (p=0.043). COX-2 expression was inversely correlating with the overall survival (r: -0.509, p=0.00245), whereas MDR1 not (r: 0.0902, p=0.611). MDR1 expression was not correlating with the survival in dogs which received chemotherapy (r: -0.282, p=0.611)

Conclusions
High COX-2 alone or with high MDR1 expression gave the lowest survival of the patients. MDR1 levels were unexpectedly unrelated with the survival times, whereas COX-2 levels were of prognostic interest in canine mammary gland tumours. The limitaton of this study is the resonably low number of cases.
Establishment of a molecular screening system for claudin-gene expression in canine neoplasias and characterisation of claudin-gene expression in canine cell lines and canine mammary tissue samples

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Introduction
A group of transmembrane proteins called claudins link adjacent cells, seal intercellular space and maintain homeostasis in epithelial tissue. Immunohistochemical studies on canine tumors show deregulation of claudin-1, -3, -4 and -7 expression. A molecular screening system for the claudin-genes -1, -3, -4 and -7 was established. Characterisation of claudin-gene-expression in canine cell lines and mammary neoplasias was performed by conventional and qPCR.

Materials and Methods
Canine cell lines: Mammary: MTH53A, non-neoplastic; ZMTH3, MTH52C: mammary tumors. Prostate: CT1258: prostate carcinoma; DT08/46: prostatic cyst. Canine mammary tissues: 10 healthy tissue samples, 49 benign neoplasia samples, 60 malignant neoplasia samples. Primers were designed for PCR and qPCR. Fragment and sequence verification was done by cloning and sequencing. RNA isolation was performed with the RNeasy® Mini Kit including a digestion of genomic DNA. cDNA-Synthesis was performed using the M-MLV Reverse Transkriptase. qPCRs analyses were performed using SYBR Green and REST2009.

Results
Conventional PCR and qPCR expression analyses: Cell lines: DT08/46: claudin-1, -3, -4 and -7 positive. MTH53A: claudin-7 positiv. ZMTH3: claudin-1 positive. CT1258, MTH52C: low to negative. The majority of mammary tissues show claudin expression in conventional PCR.

Conclusions
Using the established molecular assays the claudin-1, -3, -4 and -7 -expression of canine cell lines and mammary tissues was characterized. Thus we can refine immunohistochemical data and evaluate the diagnostic and therapeutic potential of the claudin genes in veterinary oncology.
FIRST PATIENT COHORT OF CANINE NASOSINAL TUMORS TREATED WITH RADIOTHERAPY AT THE UNIVERSITY OF VETERINARY MEDICINE VIENNA

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Introduction
Canine nasosinal tumors are a miscellaneous group of neoplasms with primarily malignant behavior. Megavoltage radiotherapy is considered treatment of choice for this tumor entity. At the University of Veterinary Medicine Vienna a linear accelerator was installed in 2006. The aim of this retrospective study was to analyse the first patient cohort treated at this institution.

Materials and Methods
Dogs which were radiated for a nasosinal tumor between 2006 and 2012 were included into the study. Medical records were reviewed and patient characteristics, treatment protocols, adjuvant therapies and outcome were analysed. Follow-up information was obtained from medical records and by phone conversations with veterinarians or pet owners.

Results
Thirty-four dogs fulfilled the inclusion criteria. Mean age was 9.1 years and mean body weight was 25.2 kg. Male dogs were overrepresented (65%) and Golden Retrievers were the most common breed. Twenty dogs were radiated curatively (mean dose 48.4 Gy) and 14 dogs palliatively (mean dose 22.6 Gy) and median survival time was 599 and 186 days, respectively. Dogs of earlier-stage disease had a significantly better outcome than dogs with more advanced disease. Adjuvant chemotherapy was used in 13 dogs. Rhinotomy was performed in four dogs after and in two before radiotherapy. Three patients had surgery earlier and were radiated with recurrent disease.

Conclusions
Outcome in this study compares favourably to the literature and confirms that radiotherapy should be recommended as standard of care for nasosinal tumors. The role of adjuvant treatment options could be further investigated in a prospective multicenter study.
EVALUATION OF THERMOGRAPHY AS A CLINICAL PROGNOSTIC FACTOR IN CANINE MAST CELL TUMORS

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Introduction
Thermography is a method used for breast cancer detection in humans, based on "hot spots". Understanding of thermographic behavior of MCT can lead us to a possible prognostic/diagnostic tool. We evaluated 15 MCT using thermographic images, histologic patterns and IHQ markers, looking for a thermographic pattern for this tumor.

Materials and Methods
Images were taken following thermographic image protocol. We measured mean temperature of central tumor point (SpT), of healthy skin away from the surgical margin (SpNT), tumoral area mean temperature (AT) and non-tumoral area (ANT). Tumors were histologic graded, AgNOR’s, PCNA, KIT and VEGF-A protein marking were studied.

Results
All tumors were MCT grade II, low grade. Mean SpT were 33.18°C (28.7-36.5). Mean SpNT were 33.39°C (28.5-36.3). Mean AT were 33.27°C (29.3-36.1) and mean ANT were 33.95°C (31.0-36.0). In 47% of the tumors the difference between the tumoral area and healthy skin was negative (tumor as a cooler point) and in 53% of the cases, the difference was positive (tumors presenting as hot point). We found no correlation between thermographyc variation with histologic patterns or IHQ markers, but we did found a statitical difference between the temperature in tumor area and healthy skin area (p<0.001).

Conclusions
Our data suggest that, besides what human literature suggest, MCT are not always detected as a hot spot on thermography. Based on our studies, we believe that thermography, can be a useful tool for diagnostic of MCT in the future, but further studies of thermographic behavior of MCT are necessary.
Canine Diffuse Large B Cell Lymphoma: serology status for canine Herpesvirus (cHV) infection and survival analysis

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Introduction
It has been described the association of gammaherpesvirus (Epstein-Barr virus) chronic infection in human lymphomagenesis. It has also been reported the presence of antibodies and viral Material in dogs affected by lymphoma. The exact role of herpesvirus in canine lymphomagenesis is still poorly understood, existing a very few data correlating cHV infection and lymphoma in dogs. The aim of this study was to evaluate serological status for canine Herpesvirus in dogs with spontaneous diffuse large B cell lymphoma.

Materials and Methods
Twenty eight dogs with diffuse large B cell lymphomas (cDLBCL) treated with a 19 week CHOP protocol were tested for cHV antibodies by a referral lab using ELISA technique. Survival analysis was then performed and correlated with cHV infection.

Results
Sixteen out of 28 (57%) of dogs revealed positive titers for cHV antibodies. Median survival time (MST) was higher in dogs suffering from DLBCL and showing negative titers for cHV (MST= 288 days) compared to those with positive titers (MST= 144 days).

Conclusions
These results may suggest serological status for cHV as a possible prognostic indicator and a tease to search for the possible role of cHV in lymphomagenesis.
Evaluation of survival time and efficacy of radiation therapy with chemotherapy for dogs with II stage osteosarcoma

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Introduction
Canine osteosarcoma (OS) is an aggressive tumor accounting for approximately 90% of all primary bone tumors in these species. Although limb amputation with chemotherapy is the gold standard of care, treatment including radiation therapy supplemented with chemotherapy can be used for limb sparing. In this study we compared radiation therapy/chemotherapy protocol with treatment without radiation therapy.

Materials and Methods
Sixteen dogs with histologically confirmed OS underwent radiation therapy (group 1) with 60 Gy of cobalt 60 radiation in 3.5-5 Gy fractionation 2-3 doses a week. All dogs received concurrent chemotherapy with either cisplatin in dose of 70 mg/m² (n=5), or doxorubicin in dose of 30 mg/m² (n=3) or combination of cisplatin (60 mg/m²) and doxorubicin in dose of 25 mg/m² (n=8).

Results
Control group 2 (n=12) underwent limb amputation with adjuvant chemotherapy (n=3) or limb-sparing surgery (n=9) with adjuvant chemotherapy without radiation therapy. Medial survival in group 1 was 4.6 mo, 1/16 dogs was alive after 1 year. In 25% of dogs was observed radiation dermatitis. Median survival in group 2 was 5.8 mo and did not differed significantly from that observed for group 1.1 year survived 1 patient.

Conclusions
Radiation therapy with chemotherapy may be considered an alternative to amputation or limb-sparing surgical procedures in selected dogs with appendicular osteosarcoma.
Second cancer incidence in patients treated with electrochemotherapy

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Introduction
Electrochemotherapy (ECT) as it is used in the veterinary field treats tumours whose recurrence risk is primarily local. The high success of the treatment in several common tumours leads to long-term survival of patients thus treated. The fact that these patients have received a cytotoxic drug could increase their risk of further malignant tumours. The purpose of this retrospective study is to assess the carcinogenic risk of electrochemotherapy using bleomycin.

Materials and Methods
This study examines retrospectively the follow-up data on 164 cases treated with electrochemotherapy for second malignancy frequency. For a control population, the records of 269 patients treated for tumours by surgery alone in first opinion practice. Data supplied from Istituto Zooprofilattico Sperimentale delle Venezie follows 8195 tumour cases in 7048 dogs; 886 dogs (10.8%) developed more than 1 tumor (Vascellari, M: personal communication).

Results
The data shows that 17/164 ECT treated (10.3%) and 60/269 surgically (22.3%) patients developed second malignancies. Where cases with >2 years follow-up are considered, 10/57 (17.5%) ECT and 38/110 (34.5%) surgical cases developed second tumours. None of the ECT treated cats developed a second neoplasm.

Conclusions
It is concluded that ECT does not increase the risk of second tumours. The nature of the control groups may not be representative, however. A similar study of patients receiving metronomic therapy would be justified.
Intra-operative Electrochemotherapy in Canine Mast Cell Tumor: Retrospective Study of 34 Cases

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Introduction
Electrochemotherapy is a new modality of local control of tumors. The intra-operative approach is an alternative to infiltrated surgical margins. The Electrochemotherapy has high selectivity neoplastic cells, allowing to treat a wide margin, without needless destruction of healthy tissue and reducing recurrence. We report 34 cases of canine mast cell tumor (MCT) treated with Electrochemotherapy intra-operative, with the aiming of achieving better local control of the disease.

Materials and Methods
Thirty-four patients with diagnosis and histopathological grading (Patnaik et al, 1984) of cutaneous MCT were treated between 2009 and 2011 and followed for 1882 days. The protocol included surgery for the tumor excision and Electrochemotherapy for treating margins, in the same procedure. All animals were staged. Tumors with II and III degree were treated with adjuvant chemotherapy.

Results
Of the 34 cases of MCT 76.5% (n = 26) showed no local recurrence and median survival was 765.5 days (46-1868). Observed mortality rate of 47%, with 35% attributed to MCT. Of the 34 cases, 25 had clinical stage IIIa and all recurrences (n = 8) occurred in this group, all of which were histological grade II (n = 6) or III (n = 2). The recurrence rate was 40% in each graduation, however, the group grade II three times larger than the third. The follow up time was defined until the writing of this paper.

Conclusions
The association of these two techniques in the treatment of cutaneous MCT may present greater chances of disease free time, lower recurrence rates and better clinical outcomes.
Canine Diffuse Large B cell Lymphoma- Survival analysis on 23 patients treated with CHOP 19 week protocol

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Introduction
In dogs the most common WHO entity seems to be Diffuse Large B cell lymphoma. Studies evaluating recent WHO lymphoma entities treatment response, as well as prognostic markers are lacking.

Materials and Methods
Twenty three cases of canine diffuse large B cell lymphoma treated with a 19 week CHOP protocol were analysed for response to treatment, time to progression and overall survival. Gender, age, weight, WHO stage, anatomic presentation and breed were assessed and correlated with prognosis.

Results
Median age of dogs was 8.87 years. Multicentric lymphoma was the most common form (87.0%). The majority of the dogs were in clinical Stage IV (73.9%). Most of the dogs (60.9%) were on substage “b”. Overall median survival time was 154 days based on the Kaplan-Meier survival analysis.

Conclusions
Our study demonstrated that distribution between genders is similar and Rottweiler breed was overrepresented. Large dogs (body weight >30 Kg) and small dogs (body weight less than 30 Kg) are equally represented and differences in survival between these 2 groups could be identified by the Long-Rank test, with small dogs apparently living less after diagnosis and treatment. No objective justification for this association could be identified. The majority of patients presents in an advanced WHO stage, mainly stages III, IV and V, respectively, with only substage b making a significant difference in worsening prognosis and consequently survival. Probably due to this reason our group of cDLBCL had a short median survival time than those reported in literature for general cL.

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Introduction

Transitional cell carcinoma is a frequent type of canine urinary bladder cancer. Medical treatment with chemotherapy, COX inhibitor has been the mainstay of treatment for dogs with TCC. Surgery is rarely suggested, because it’s located trigone region and has a high incidence of metastases.

Materials and Methods

From 2009-2013, 27 dogs with clinical signs compatible with urinary bladder cancer were identified.

Results

The most frequent breeds were 7 Poodle, 6 Mixed Breed, and the remainder included 3 Cocker and 3 PitBull, 2 of each Bernese and LhasaA, 1 of each Dachshund, Fox, Pinscher and ScottishT. 16 (59%) were female and 11 (41%) male. The mean age was 10.5 years (median: 12, range: 2-15) and the mean weight was 12 kg (median: 8, range: 3.3-48). The clinical signs consisted of hematuria (68%), dysuria (46%) and pollakiuria (21%). The mean duration of clinical signs before diagnosis was 3 months (median: 2, range: 1 week to 8 months). The mean size of the tumor measured by ultrasound were 2.9 cm (± 1.7) and the most common location were trigone (n=14=52%), cranial (n=6=22%), lateral (n=3=11%), whole wall (n=2=7%), dorsal (n=1=4%) and ventral bladder wall (n=1=4%). Diagnosis was obtained by esfoliative cytology (catheterization) in 19 dogs, revealing TCC in 15 (79%) dogs and benign epithelial desquamation in 4 (21%). Dogs submitted to surgery had the histopathologic confirmation of TCC.

Conclusions

The average survival time was correlated with the treatment: 106 (±78) days the group which had been treated only with piroxicam; 217 (±201) days piroxicam with carboplatin; 225 (±105) days surgery associated with piroxicam and 240 (±252) days surgery associated with piroxicam and carboplatin.
Definition of a Ki-67 threshold for canine B-cell lymphoma

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Introduction
B-cell lymphomas (BCL) are heterogeneous in terms of epidemiology, immunophenotype and outcome in dogs. We have recently shown that immunohistochemistry (IHC) for CD10, BCL-6, MUM-1, BCL-2 and c-MYC, using Hans’ algorithm derived from human medicine, was of prognostic value in canine diffuse large B-cell lymphomas (DLBCL) (Nguyen et al. 2013). In this study, we evaluate the prognostic value of another IHC marker, Ki-67, in canine BCL.

Materials and Methods
253 BCLs diagnosed using histopathological criteria (Valli et al. 2011) and IHC (CD3-, CD20+ and CD79acy+) at Oniris, treated by surgery (8%), corticosteroids (53%) or CHOP-like chemotherapy (28%) or untreated (11%) and with an available 2-year follow-up period, were evaluated for Ki-67 by automated IHC.

Results
The BCL were DLBCL (51%), marginal zone lymphomas (30%), follicular lymphomas (4%), other high grade (9%) or low grade BCL (6%). The histological grade was of prognostic value (median specific survival of 51d for low grades, of 143d for high grades) (p=0.014). The optimal Ki-67 threshold for BCL was established at 40%, with correlation to survival (p<0.001, ANOVA test) and separating low and high grade BCL with 88% sensitivity and 100% specificity (positive and negative predictive values of respectively 100 and 93%).

Conclusions
We recommend the threshold of 40% for Ki-67 in canine BCL to better identify dogs having a longer survival potential. This threshold is similar to the one defined for human lymphomas (Naz et al. 2011).
TOCERANIB PHOSPHATE, CYCLOPHOSPHAMIDE AND PREDNISONE AS A RESCUE PROTOCOL IN CANINE MULTICENTRIC LYMPHOMA

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Introduction
Vascular endothelial growth factor (VEGF) is expressed both in vitro and in vivo in human and canine lymphoma. Antiangiogenic treatment regimens have shown significant clinical activity in recurrent NHL in human medicine. The objective of this study was to prospectively evaluate an antiangiogenic treatment protocol in the setting of relapsed canine multicentric lymphoma.

Materials and Methods
Inclusion required a cytologic or histologic diagnosis of relapsed multicentric lymphoma. Any prior therapy was allowed. The protocol consisted in: Cyclophosphamide at 15 mg/m^2/24h PO, toceranib phosphate at 2.5 mg/kg Monday, Wednesday and Friday, and prednisone at a tapering dose starting at 2mg/kg/24h, to then in one month time continue with 0.5mg/kg/24h PO. Responses were evaluated according to the Veterinary cooperative oncology group’s (VCOG) response evaluation criteria for peripheral nodal lymphoma in dogs (v1.0). Outcome was measured as time to progression. Adverse events were graded according to the Veterinary cooperative oncology group’s common terminology criteria for adverse events (VCOG-CTCAE).

Results
Ten dogs were enrolled. Immunohistochemistry was available in 5 cases (4 B-cell, one T-cell lymphoma). Four dogs (40%) achieved stable disease, with a median time to progression of 46 days. No partial or complete responses were observed. Four dogs experienced gastrointestinal upsets (grade II n= 2, grade III n=2), two developed neutropenia (grade I n=1, grade II n=1). Toceranib was discontinued in one case during 5 days.

Conclusions
This protocol was well-tolerated, but lacked overt benefit in advanced canine lymphoma. The protocol may have greater activity in specific sub-classifications of canine lymphoma, or earlier in the disease process.
PROSPECTIVE STUDY OF TOCERANIB PHOSPHATE (Palladia®) IN FELINE MAMMARY TUMORS

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Introduction
Vascular endothelial growth factor (VEGF) is overexpressed in feline mammary tumors and it is associated with worse prognosis. Toceranib phosphate (Palladia®), a tyrosine kinase inhibitor (VEGFR, PDGFR, KIT) has been used safely in tumor bearing cats. This prospective study evaluated the efficacy and safety of toceranib phosphate in cats with measurable mammary tumors.

Materials and Methods
Inclusion criteria included a histopathological diagnosis of primary or metastatic mammary carcinoma. The initial assessment included physical exam, tumor measurements, CBC, biochemistry and UA, thoracic radiographs and abdominal ultrasound. Toceranib phosphate dose was 2.5-3.25 mg/kg/48h. A CBC and physical exam were performed at two weeks time, response evaluation following RECIST criteria was performed one month after initiating the protocol and monthly thereafter. Adverse events were recorded and evaluated following VCOG-CTCAE.

Results
Sixteen animals have been included until abstract submission. Five patients presented with surgical recurrence, 3 had distant metastatic disease, and 8 were “de novo” tumors. One cat had a complete response, 6 had partial responses, 4 stable disease and 5 animals had progressive disease. Two patients developed grade I neutropenia, one grade II vomiting, one grade III anorexia and a one grade II azotemia. Total response rate was 50%, median time to progression of 98 days and clinical benefit of 68%.

Conclusions
Treatment was well tolerated with minor side effects. These data provides evidence that toceranib exhibits activity in feline mammary tumors in the macroscopic setting, suggesting a potential role in the adjuvant microscopic setting after surgery. Future prospective studies are needed to study this possibility further.
CYCLOOXYGENASE-2 EXPRESSION IN EQUINE CUTANEOUS NEOPLASMS

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Introduction
Cutaneous neoplastic lesions are very common in horses, accounting for 50% of all tumours that affect these animals. Retrospective studies have described sarcomas, squamous cell carcinomas (SCC) and melanocytic tumours as the most common lesions. The purpose of this study was to analyze, through immunohistochemistry, COX-2 expression in various equine cutaneous tumours, in order to determine whether COX-2 is a potential therapeutic target.

Materials and Methods
Thirty-five tumour samples were subjected to histopathological diagnosis, according to the criteria described by WHO. The material selected included 24 melanomas, 5 sarcomas, 4 SCC, 1 papilloma and 1 fibroma. Pigmented lesions were bleached, for melanin removal, previously to immunohistochemical procedures. Immunohistochemical study was performed using the clone SP21 (ThermoScientific®), diluted in 1:50. COX-2 expression was assessed according to its labelling extension and intensity.

Results
COX-2 was expressed in 86% (n=30) of the samples studied, scored positive in 20 of 24 (83%) melanomas, 3 of 4 (75%) SCC, in 4 of 5 (80%) sarcomas and in both papilloma and fibroma. In positive tumours, neoplastic cells always demonstrated a cytoplasmatic labelling pattern, some with an increased intensity in perinuclear region. 60% (n=21) of the COX-2 positive tumours had a diffuse immunoreexpression, with extension superior to 50% of the cells. Regarding the labelling intensity, melanoma and sarcomatoid samples got equally distributed through the three different intensity grades. 100% of the COX-2 positive CCE presented a strong labelling intensity.

Conclusions
In summary, most equine cutaneous neoplasms appear to express COX-2, therefore eligible for treatment and prevention by the use of COX-2 inhibitors.
BIOLOGICAL CHARACTERIZATION AND SENSITIVITY TO METFORMIN OF CANCER STEM-LIKE CELLS FROM CANINE OSTEOSARCOMA CELL LINES

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Introduction
Cancer stem cells (CSCs), identified in many human cancers including osteosarcoma (OSA) are drug resistant and, likely, play a major role in tumor recurrence and metastasization, thus representing candidate drug targets. The aim of this study was to isolate and characterize CSCs from canine OSA cell lines, evaluating their responsiveness to metformin, an anti-diabetic drug, that possesses antitumor properties and affects CSC survival in different human tumors.

Materials and Methods
CSCs isolated from 2 canine primary cultures (one osteoblastic productive and one chondroblastic OSA), were enriched in stem-permissive medium and characterized for proliferative potential and sensitivity to metformin by MTT assay, self-renewal by spheroid forming-assay, differentiation ability and stem marker expression by immunofluorescence. Tumorigenicity was tested by s.c. injection of cells into NOD/SCID mice.

Results
We identified and enriched CSCs maintaining OSA cells in a medium containing EGF/bFGF. The free-floating sarcospheres expressed stem cell markers CD133, Oct4 and CXCR4, were able to self-renew and, can be expanded for several passages and gave rise to differentiated adherent monolayers in serum-containing medium. Injection of CSCs into mice resulted in the formation of tumors that recapitulated the features of canine OSA. Finally, metformin induced a significant antiproliferative effect on CSCs.

Conclusions
Our results indicate that canine OSA contains a subpopulation of CSCs sensitive to metformin and may represent a novel experimental model for pre-clinical in vitro drug efficacy screening, further confirming the potential for the dog as a model of human tumors.
P-cadherin expression as a hallmark of malignancy in feline mammary tumours

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Introduction
Classical cadherins, E-cadherin (epithelial) and P-cadherin (placental), are the best characterised members of the cadherin superfamily. Changes in their expressions have been related to breast carcinogenesis, however their role in feline mammary tumours is still poorly understood. The purposes of this study were to examine the expression of P-cadherin in feline spontaneous mammary lesions and to determine its relationship with the expression of E-cadherin and clinicopathological characteristics with known prognostic value.

Materials and Methods
The expressions of P-cadherin and E-cadherin were studied by immunohistochemistry in a series of feline normal mammary glands, hyperplastic/dysplastic lesions, benign and malignant tumours, and related with variables with known prognostic value, such as histological grade, mode of growth, tumour largest diameter, presence of necrosis, skin ulceration, lymph node metastases, and neoplastic intravascular emboli.

Results
In normal tissues and in the majority of hyperplastic/dysplastic lesions and benign tumours, P-cadherin was restricted to myoepithelial cells, while 80% of the malignant tumours expressed P-cadherin in the luminal epithelial cells. Furthermore, this pattern was significantly related to high histological grade, infiltrative growth, tumour necrosis and presence of neoplastic emboli. The combined P- and E-cadherin expression profiles of high grade and infiltrative tumours were very similar, the large majority expressing P-cadherin with an almost equitable distribution of preserved and reduced E-cadherin expression among them.

Conclusions
The present study demonstrated a relation between the aberrant expression of P-cadherin and a malignant phenotype, higher histological grade and invasive behaviour, suggesting it as an indicator of poor prognosis in feline mammary tumours.
Effects of toceranib phosphate on canine osteosarcoma cell lines

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Introduction
Osteosarcoma (OSA) is the most common canine primary bone tumour. Understanding the expression and role of tyrosine kinase receptors (TKRs) in OSA is important in order to find innovative therapeutic strategies. The aim of this study was to evaluate TKRs expression and assess the effect of toceranib phosphate in seven canine OSA cell lines.

Materials and Methods
VEGFR2, C-Kit and PDGFRα/β expression (main targets of toceranib phosphate) was assessed by quantitative PCR in seven primary canine OSA cell lines and normal osteoblasts. Cell cycle, cell proliferation, survival, motility, invasion and soft-agar assays were carried out in absence or presence of toceranib phosphate. Cell lines tumorigenicity was tested in vivo.

Results
PDGFRα and PDGFRβ were highly expressed in all cell lines. VEGFR2 and C-Kit were overexpressed in 3 cell lines (Penny, Wall and Lord), in which toceranib phosphate did not affect Ki67 cell proliferation, cell cycle and survival. All cell lines showed migratory ability in both transwell and wound healing assays that was inhibited (26%-93%) by toceranib phosphate. Only Penny and Wall cell lines were able to form soft-agar colonies that toceranib phosphate decreased by 38% and 86%, respectively. Penny, Wall and Lord developed tumors in mice. PDGFRα/β and VEGFR2 expression was demonstrated by immunohistochemistry

Conclusions
All OSA cell lines expressed PDGFRα/β, while VEGFR2 and C-Kit were expressed in 3/7. Toceranib phosphate caused inhibitory effect in the migration and anchorage-independent cell growth in all cell lines. Three OSA cell lines developed tumours that maintained the original immunohistochemical characteristics.
APOPTOSIS AND PROLIFERATION IN CANINE MAMMARY TUMORS

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Introduction

The rate of tumor growth is determined by tumor cell proliferation and death. An imbalance between tumor cell proliferation and death is critical for tumor development, progression and regression. The goal of the present study is try to elucidate the relationship between tumoral proliferation and apoptosis in canine mammary tumours (CMT).

Materials and Methods

Samples of 50 CMT (18 benign and 32 malignant) were studied, by immunohistochemical detection of active Caspase-3 and Ki-67 antigens in order to determine its association with several clinic and pathological parameters.

Results

Caspase-3 immunoexpression was statistically significantly higher in benign tumors comparatively to malign counterparts (p ≤ 0.001). In the malign tumors’ group, there was no significant association between active Caspase-3 and the clinic-pathological variables considered. The Ki-67 immunoexpression was statistically significantly higher on malignant tumors comparatively to benign ones (P ≤ 0.001). In the malignant tumors’ group, the Ki-67 PI show a statistically significant association with the tumor size (p = 0.025), histological type (p = 0.010), mitotic index (p ≤0.001), nuclear degree (p = 0.025), histological grade of malignancy (p =0.002) and presence of metastases at regional lymph nodes (p = 0.025). Furthermore, in this study was observed a negative correlation between the Ki-67 PI and the expression of the active Caspase-3 (r = -0.034; p = 0.049).

Conclusions

Our results highlighted the role of Ki-67 in mammary carcinogenesis and tumor progression. With regard the active Caspase-3 our results suggest that apoptotic pathways are inhibited in tumor progression.
Inhibition of telomerase in canine sarcoma cell lines reduces tumor cell viability

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Introduction
Tumor cells bypass replicative senescence by activating telomere maintenance mechanisms (TMM). Activation of telomerase is the most commonly used TMM. Thus, inhibition of telomerase is an attractive anticancer strategy. The objective of this study was to screen different telomerase inhibitors for their potential to impair canine soft tissue sarcoma cell line viability.

Materials and Methods
Three telomerase positive canine fibrosarcoma cell lines (PSTS, CoFSA, MBSa) and three human control cell lines of varying origin (sarcoma, carcinoma and glioblastoma) were used for this study. Telomerase activity (TA) was modulated by infection with recombinant adenovirus (AV) expressing either human wild-type (activation) or dominant-negative (inhibition) catalytic subunit of telomerase. Further TA was inhibited with MST-312, BIBR1532 or TMPyP4, a quadruplex ligand. TA was determined by qPCR-TRAP, tumor cell viability by MTT and tumor cell senescence by acidic β-galactosidase assay.

Results
Expression of wild-type telomerase caused further activation of TA, whereas expression of dominant-negative telomerase attenuated TA in 2/3 canine sarcoma cell lines, but induced cell senescence in all. MST-312 and BIBR1532 both impaired TA, but MST-312 was more potent inhibiting tumor cell viability (IC\(_{50}\) 0.78-5.1µM). BIBR1532 required higher drug concentrations (IC\(_{50}\)s 43.2 and 53.1µM) and could only inhibit 2/3 cell lines. TMPyP4 seemed least effective at repressing tumor viability with IC\(_{50}\) of 60µM.

Conclusions
In conclusion, TA can be impaired in canine sarcoma cell lines with different inhibitors, but MST-312 caused the most potent decrease in tumor cell viability. This drug appears promising to further investigate its potential for targeted anticancer therapy in aggressive sarcomas.
Evaluation of patent blue dye for sentinel lymph node identification in canine mammary tumours

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Introduction
The colorimetric and/or isotopic “sentinel” lymph node (LN) procedure has replaced axillary LN dissection for staging of clinically N0 human patients with breast cancer. Despite the prognostic value of LN metastatic status, the usefulness of vital dyes injection for lymphatic mapping has not been extensively evaluated in veterinary oncology. Because of the variations observed in the mammary lymphatic network and the difficulty of axillary LNs surgical removal, it is hypothesized that better visualisation of local lymphatic drainage would be helpful for mammary tumour (MT) surgery.

Materials and Methods
Fifteen minutes before the mastectomy, 2ml of BLEU PATENTE V 2,5% was injected subcutaneously at the direct periphery of the MT on 10 anaesthetized female dogs, with owner’s consent. A small incision was performed in the axillary region for resection of the axillary LN(s). The surgery ended with the removal of the inguinal LN. The blue coloration was evaluated during mastectomy and on removed tissues.

Results
MT (60% malignant) were located on M2 (25%), M3 (33%), M4 (25%) and M5 (17%). Clinical evidence of toxicity was absent but transitory unwanted staining of non-lymphatic tissues and of excreta (mostly urine) was frequent. The blue dye failed to stain locally in one female dog. Successful staining was more frequent for the inguinal LN than for the axillary LN, which was not systematically detected by the surgeon (60%). Tolerance of the procedure was excellent but induced a blue coloration of the surgical operating field, routinely tolerated in human surgery.

Conclusions
This promising technique needs further optimisation on a larger series.
Factors and transporters affecting 5-ALA PDT in tumor-bearing cats

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Introduction
Photodynamic therapy (PDT) is an alternative modality to treat certain types of tumors, and is based on production of free radicals (FR). Patients are administered a harmless light-sensitive drug, and after its accumulation in the tumor, the affected area is illuminated with visible light, so cytotoxic ROS are produced in a strictly located area, leading to tumor destruction.

Materials and Methods
A precursor of a light-sensitive drug, 5-aminolevulinic acid (5-ALA) has been administered to cats with tumors. 5-ALA is converted to Protoporphyrin IX in cells during haem production, and has excellent photodynamic properties, especially when overproduced. PDT takes advantage of ROS production, so mRNA levels of: (i) HIF1-α and HO-1 factors, as well as COX2 enzyme (related to oxygen consumption), (ii) and porphyrin-related transporters (ABCG2, ABCB6 and PEPT1) have been examined during PDT. In vivo production of Protoporphyrin IX and FR were also measured in tumor tissues using fluorescent and EPR spectroscopies, respectively. PDT efficacy was assessed based on RFP (recurrence-free period).

Results
Efficiency of PDT correlated with COX-2 (R = -0.74), HO-1 (R = -0.70) and PEPT1 (R = 0.69) expression of tumor tissues. Grade of tumor progression was also important, and had direct correlation with FR production (p = 0.014).

Conclusions
PDT efficacy is not related as much to tumor type, as to its grade and oxygen-related factor expressions. Among 5-ALA-PDT-related transporters, the most important is the one, that provides tumor cells with the protoporphyrin precursor. Other transporters taking part in the process of photosensitizer metabolism do not seem to be determinant.
Toceranib and COX2 inhibitor as palliative treatment or adjuvant to conservative surgery in dogs with oral melanoma

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Introduction
Canine melanoma (OM) is a very invasive tumour that represents the most common malignancy in the oral cavity. The objective of this retrospective study was to evaluate the clinical benefit, the median survival time (MST) and the adverse effects (AE) in dogs with OM, treated with antiangiogenic drugs as unique therapy or adjuvant to conservative surgery.

Materials and Methods
Seventeen dogs with OM were treated with toceranib (Palladia®) and COX2 inhibitor +/-metronomic therapy. Seven received this combination as unique therapy and ten as adjuvant after conservative surgery. Clinical benefit (defined as complete or partial response and stable disease) was evaluated on days 30, 60, 120, 180, 240, 300, 360. Adverse events were registered following the VCOG-CTCAE criteria.

Results
Clinical benefit was observed in 4/7 (57%) dogs treated with toceranib and COX2 inhibitor as palliative treatment; however it was a short response. Dogs treated with toceranib and COX2 inhibitor +/- metronomic therapy as adjuvant to conservative surgery (n=10) have a MST of 530 (±145) days and 2/10 dogs (20%) are still alive 1 year after the diagnosis. Otherwise, MST in the arm of dogs treated with the palliative modality was 85 (±19) days. AE were reported in only 2/17 (11.8%).

Conclusions
Most of the dogs tolerated very well this therapy which seems promising when used as adjuvant to conservative surgery. The effectiveness looks comparable to that previously reported with the classical radiation/chemotherapy approach. Further prospective trials are needed to determine more accurately the impact of this therapy in canine oral melanoma.
HEMATOLOGY RESULTS OF DOGS WITH LYMPHOMA AT INITIAL PRESENTATION

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Introduction
Few publications have evaluated the prognostic value of hematologic abnormalities at time of diagnosis of canine lymphoma. Monocytosis, lymphopenia and neutrophil/lymphocyte ratio >3.5 and anemia were identified as negative prognostic factors. Objective of this study was to determine the nature and frequency of hematologic abnormalities in newly diagnosed canine lymphoma. In a retrospective study the initial CBCs of 226 dogs with confirmed lymphoma were evaluated.

Materials and Methods
CBC was performed by an ADVIA™ system, manual differentials were performed, when a numerical abnormality exceeded 25% of the respective cut-off and/or scattergram abnormalities were present.

Results
69% of the patients showed monocytosis (>0.5x10³/µl), 56.6% an N:L ratio >3.5, 44.7% a leukocytosis (>15x10³/µl), 39.4% neutrophilia (>11.25x10³/µl), 37.2% combined leukocytosis and neutrophilia. 22.6% were anemic (Hct<37%, RBC<5.5x10⁶/µl, Hgb<12g/dL). Lymphocytosis (>4.5x10³/µl) occurred in 15.9%, leukocytosis combined with lymphocytosis in 15%. Leukopenia (<6x10³/µl) was seen in 10.2%. The frequency of neutropenia (<3.3x10³/µl) was 8.4%, 7.1% for thrombocytosis (>500x10³/µl) and lymphopenia (<0.78x10³/µl) in 4.9%. PLT counts were available in 168 patients and 25.5% showed thrombocytopenia (<150x10³/µl). In 116 patients differentiation into B and T-cell lymphoma was performed. T-cell lymphoma patients had a higher frequency of leukopenia (16.7%) and neutropenia (19%) whereas B-cell lymphoma patients showed leukocytosis (43.8%). Anemia was more often recorded in B-cell lymphomas (Hgb<12g/dL in 32.6%).

Conclusions
In this cohort monocytosis and a high N:L ratio were present in more than 50% of cases. Their significance in terms of prognosis in canine lymphoma has to be investigated as well as the differences encountered between B- and T-cell lymphoma.
c-Kit immunoexpression pattern in melanotic and amelanotic canine oral melanomas

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Introduction
The c-Kit is a tyrosine kinase receptor, which aberrant activation is implicated in a variety of tumors. Although c-Kit activity is critical to melanocyte development and migration, its expression tends to be lost in most melanomas, mainly in the invasive and metastatic types. This study aimed to analyze the c-Kit protein expression in melanotic and amelanotic melanomas from oral cavity of dogs.

Materials and Methods
A total of 34 melanomas were collected, being 19 melanotic and 15 amelanotic specimens. All samples were immunostained for Melan-A, HMB45, and c-kit (Dako, rabbit antibody). Number of positive cells were randomly counted.

Results
Amelanotic melanomas grew faster and presented a higher incidence of metastasis. The epithelioid melanoma was the most common morphologic type among the melanotic melanomas, but among amelanotic melanomas, the same number of epithelioid and spindle types were obtained. The results have shown a greater number of c-Kit positive cells in amelanotic tumors and a predominant cytoplasmic location of c-Kit in melanotic tumors. The intensity of c-Kit immunolabeling was mostly moderate in melanotic melanomas and mostly strong in amelanotic melanomas.

Conclusions
These results indicate a potential role for c-Kit in the canine oral melanomas with clear differences in expression and location patterns between the two histological types of the tumor – melanotic and amelanotic. This study highlights the importance of a detailed study of c-Kit mutations in canine oral melanoma to better understand the molecular mechanisms implicated in the development of this disease, permitting a more functional classification of the patients into potential therapeutic classes.
Quality of life evaluation in cancer patients using the HHHHMM® scale

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Introduction
The use of various evaluation scales helps prevent complications associated with the tumor or tumor therapy and / or to implement targeted therapeutic adjustments to improve the quality of life of the cancer patient. The objective of this study was to evaluate the implementation and subsequent use of information obtained about quality of life in cancer patients initiating chemotherapy, based on the adapted scale Villalobos, AE, Quality of Life Scale helps make the final call.

Materials and Methods
The scale was provided to the owners of the patients and they were asked to rate from 1 (worst) to 10 (excellent) 7 items on quality of life of his animal, such as: Hurt, Hunger, Hydration, Hygiene, Happiness, Mobility, and “More Good Days than Bad.” The values obtained in each plot were summed and when more than 35 were considered positive. The sample included 20 patients (canine and feline) of both sexes evaluated during the first week after initiation of chemotherapy.

Results
All items showed, in average, positive results (above 5), being “Hygiene” the highest with 8.6. The average value for quality of life of the patients was 54, therefore positive (above 35).

Conclusions
All items showed positive results, meaning that all strategies implemented like analgesia protocols and more palatable diets helped.
Epidemiology of canine mammary tumours: individual risk factors and similarities to human breast cancer

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Introduction
Mammary tumours (MT) are the most common type of tumour in female dogs, but little information about their incidence in dog population is available. Breast cancer is also one of the most common cancers in women and there are some epidemiological and biological similarities between canine and human mammary tumours.

Materials and Methods
The incidence rate (IR) of MT in female dogs, living in Venice and Vicenza provinces (Northeastern Italy), during 2005-2013 were calculated as annual rate per 100,000 animals. Tumours were classified according to the WHO International histological classification of tumours of domestic animals. Data about sex, breed, and age were employed in the determination of the structure of the canine population to obtain specific incidence rates.

Results
Overall 2744 mammary tumours were reported, accounting for 54% of all tumours in female dogs. The annual IR for malignant tumours was 150 (95%CI: 142.12 – 156.67). Keeping in consideration the spayed status, the 26% of cases were detected in neutered subjects. The mean age at diagnosis resulted significantly higher for neutered dogs (10.09 years) than for entire females (9.20 years). The Samoyed, Dobermann, Schnauzer and Yorkshire Terrier dogs resulted to be more incline to develop malignant neoplasms than the overall population average.

Conclusions
In Veneto region, breast cancer is the most frequent tumour in women, with an IR of 176 new cases per 100,000 females. It is worth noting that the IR observed in canine population is similar to that of humans, supporting the validity of canine MT as epidemiologic model for human breast cancer.
Squamous Cell Carcinoma in Cat: Retrospective Study of 32 cases of head and neck localization

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Introduction
Squamous cell carcinoma is the most common skin tumor in cats affecting animals of all ages, being most frequently diagnosed in geriatric and clear coat cats. The clinical presentation can range from small superficial to deep infiltrative lesions. Therapeutic approaches include surgery, which may be followed by photodynamic (PDT) and/or cryotherapy, depending on the clinical stage at diagnosis.

Materials and Methods
The aims of this study were retrospectively evaluation of the clinical outcome, and therapeutic response in 32 cases of squamous cell carcinoma diagnosed in cats selected between 2008 and 2013.

Results
Of the 32 cases studied, 62.5 % (20/32) had stage 1 at diagnosis, and 37.5 % (12/20) had stage 2. Two cases (6.3 %) with regional lymph node metastasis were identified. Twelve out of 32 cases (37.5%) were subjected to photodynamic therapy. The median survival time (MST) for the sample studies was 818 days. Regarding patients submitted to photodynamic therapy, MST was 940 days, a higher result compared to those who were not submitted to PDT (MST 744 days).

Conclusions
The results should be interpreted cautiously, given the existence of several variables, such as surgical margins and different chemotherapy protocols previously used. However, these results reveal the relevance of adjuvant therapies, alternative or even neoadjuvant to surgery and its contribution to the reduction of relapse. According to our results PDT revealed to be one of the best treatment options for cats with squamous cell carcinoma. However further prospective and randomized studies are required to confirm our results.
Electrochemotherapy in canine squamous cell carcinoma (SCC) disseminated in medial abdomen and pelvic limbs: Case Report

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Introduction
The Electrochemotherapy (ECT) is a technique for local control of neoplasm that has been gaining ground among the cancer treatments, because of its high rate of objective response to neoplasms of various histological types, beyond the simplicity of execution and low toxicity. We reported a case of SCC committing wide and deep one of the pelvic limbs of a dog and treated conservatively with multiple electrochemotherapy sessions, resulting in complete remission.

Materials and Methods
The formation has 20cm in diameter, on the medial face of the left hind limb, extending from the inguinal region to the distal femur portion. Numerous other verrucous lesions, ranging from 0.3 cm to 2.5 cm in diameter, presented themselves throughout the abdominal region and extending to the medial face of the right hind limb, the region of distal femur. The inguinal lymph node was involved. In the beginning of the treatment Clinical staging of the patient was T4N3M0. Other clinical signs were not observed. Eight ECT sessions were held over a period of 164 days, at intervals ranging 14-37 days. In each session, the animal underwent general anesthesia. Biopsies were performed in order to confirm the presence of tumor cells prior to the ECT. For this purpose, the technique of cryotomy was used.

Results
After the ECT sessions, the animal showed complete remission of the disease. Until the writing of this paper the animal was disease free, totaling 373 days.

Conclusions
The results indicate the potential of ECT as an excellent tool to SCC conservative control of advanced clinical stage
Oxidative Status in Feline Malignant Mammary Tumours

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Introduction
Oxidative status is dependent of balance between oxidants and antioxidant defences. Oxidative stress impairs organic function and is associated to cellular senescence and death, and has been associated with various feline diseases. The aim of this study was to characterize the oxidative status in cats with spontaneous malignant mammary tumours.

Materials and Methods
Serum total antioxidant capacity (TAC) and paraoxonase-1 (PON-1) were determined in 20 female cats with malignant mammary tumours. Cats with history of previous tumours or with concomitant tumours or other diseases were excluded. Information on tumour type, histological grade, size, location, ulceration, vascular neoplastic infiltration, necrosis, metastases and survival time from diagnosis was assessed. Blood samples were collected before surgery in all cats, and whenever possible, serial samples collected on control visits.

Results
Studied population included 20 domestic short-haired cats with mammary carcinomas - solid carcinomas (n=10), tubulopapillary carcinomas (n=8), one cribriform carcinoma and one carcinosarcoma.

In the studied population, serum PON-1 ranged from 1.83-5.99 IU/ml, mean 3.72 IU/ml, standard deviation 1.07 IU/ml (reference range 3.0-4.3 IU/ml), and serum TAC ranged from 0.0-0.51 mmol/l, mean 0.36 mmol/l, standard deviation 0.17 mmol/l (reference range >0.35 mmol/l), at the time of surgery.

The serum concentrations of TAC were significantly associated with presence of tumour ulceration (p=0.033) and vascular neoplastic infiltration (p=0.022). Development of thoracic metastases was significantly associated with a decrease of serum PON-1 (p=0.021).

Conclusions
This study suggests that development of tumour ulceration, vascular infiltration with neoplastic trombi and thoracic metastases in cats with malignant mammary tumours are associated with oxidative stress.
Published only
Clinical outcomes of different treatment combinations in canine melanoma patients in Hungary

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Introduction
Treating the oral as well as the skin malignant melanoma in canine patients is still one of the major challenges for veterinary clinicians.

Materials and Methods
Altogether 69 oral or skin malignant melanoma bearing dogs were referred into our Institute for postsurgical treatments. Treatment groups were: Group I (radiation + medical 22 dogs), Group II (radiation 20 dogs), Group III (medical 12 dogs, Group IV (alternatives 15 dogs). Recurrence time, survival time, side effects and owner-satisfaction were evaluated by treatment groups.

Results
Only 3 dogs were alive two years after initiating the postsurgical treatments all the 3 belong to Group I. Four dogs were anaesthetized because of unrelated reasons and we lost 7 patients during the follow-ups. The median progression-free survival and the overall survival time was 495 and 560 days in Group I, 445 and 502 days in Group II, 414 and 461 days in Group III, and 128 and 210 days in Group IV, respectively. Dogs tolerated well the local irradiations and systemic carboplatin treatments. There was only a single case in Group I, where local irradiations must be stopped after the 8th fraction because of mucosa ulceration and two further cases both in Group III, because of decreasing blood cell numbers and consequent infections.

Conclusions
The median progression-free survival and overall survival time showed significantly lower data in Group IV compared to Group I and II but in case of Group III the difference proved to be non-significant. Neither we could make statistical differences among the data of Groups I, II, and III.
Long term efficacy of metronomic oral therapy in a case of non-resectable sublingual SCC

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Introduction
Squamous cell carcinoma (SCC) is a common lingual neoplasia in dogs. Although surgical resection is recommended, sometimes this surgical treatment is not possible and radiotherapy and/or medical treatment could be the only treatment choice. Here we report a case of sublingual SCC that was treated with oral metronomic therapy with a very good long term response.

Materials and Methods
A 15 year old male mixed-breed dog was presented with a history of hiporexia and halitosis. An ulcerated solid sublingual mass was detected in the base of the tongue. Histopathological examination of the mass revealed infiltrative SCC. FNA of regional lymph nodes and thorax radiograph did not reveal any metastasis. Due to localization and extension of the tumor, surgical excision was declined by the owner and metronomic oral therapy (toceranib phosphate 2.5 mg/kg/48h + cyclophosphamide 10 mg/m²/48h + firocoxib 5 mg/kg/24h) was established.

Results
Three months after initiating therapy the mass was greatly reduced and there was no ulceration, the dog recovered his appetite and had no problems taking food. Currently, more than 1 year after diagnosis, the dog is still receiving this oral therapy, and is doing well, no signs of disease are shown. As side effects, only two episodes of diarrhoea that were resolved with symptomatic treatment and in one occasion with toceranib interruption for 1 week have been observed.

Conclusions
Continuous oral metronomic chemotherapy (receptor kinase inhibitor + low dose alkylating agent + COX2 inhibitor) can be very effective for treating dogs with oral SCC when surgical excision or radiotherapy are not feasible.
Adjuvant Treatment with Toceranib Phosphate and Metronomic Chemotherapy on a Cat with Aggressive Mammary Tumor


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Introduction
Feline mammary tumors are the third most common type of feline neoplasia. Conventional treatment for cats with mammary tumors is radical mastectomy with or without adjuvant doxorubicin, but to our knowledge there is no definitive evidence of clinical benefits of adjuvant chemotherapy. We report a case of a cat with a highly malignant mammary tumor treated with radical mastectomy plus toceranib phosphate and metronomic chemotherapy.

Materials and Methods
A twelve-year-old female cat was presented with multiple nodular lesions around a surgical scar of a previous mastectomy of the fourth left mammary gland. There was no evidence of distant metastasis on radiological or ultrasound examination. A radical mastectomy was performed and the tissue was submitted for anatomicopathological examination. Tumour was histologically diagnosed as simple tubular-papillary carcinoma grade III with signs of vascular invasion. Three weeks after surgery, once the surgical wound was totally healed, the cat started antiangiogenic therapy with toceranib phosphate (3.33 mg/kg every 72 hours) and cyclophosphamide (10 mg/m² every 48 hours).

Results
After seven months of treatment and twelve months after the first surgery, no hematological abnormalities were detected and tolerance to both drugs was excellent. No evidence of local recurrence or distant metastasis was noted on the subsequent physical examinations and thoracic radiographs.

Conclusions
In this case, antiangiogenic therapy with toceranib phosphate and cyclophosphamide was a good adjuvant option to treat a feline patient after mammary carcinoma resection, resulting in the control of the disease with an excellent quality of life for the animal.
Electrochemotherapy as alternative treatment in a lower eyelid carcinoma in a cat

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Introduction
Electrochemotherapy is an alternative, not invasive treatment to control local neoplasia. It combines electroporation (The making of reversible porosity of cell membranes by short electric pulse) and chemotherapeutic agent as bleomycin, systemically administered.

Materials and Methods
The eyelid carcinoma may have metastatic potential and local invasiveness and "en bloc" excision with surgical reconstruction of eyelid is required. The adverse effects of surgery may be epiphora, corneal lesions, hair growth from the upper margin of the skin flap, recurrence of neoplasia.

Results
A 10 years old male natured cat was referred for lower eyelid mass. Cytological analysis was suggestive of medium/high grade carcinoma. The cat was staged with a complete blood cell count, serum biochemical profile and Tc totalbody. The neoplasia was treated by ECT in general anesthesia. The electric pulses was generated by Ciniporator, IGEA (8 square wave pulses 1000 V/cm for 100µs at 5kHz) using linear needle electrodes. The neoplasia and its lymphnodes was electroporated 10 minutes after the systemically administration of bleomycin (15 UI/m2).

Conclusions
A lower eyelid carcinoma was treated by ECT, without pain or any complications afterwards. After 240 days, the cat continued in total remission with satisfactory cosmetic effect and preservation of palpebral andnexas. The ECT is a good alternative method to the surgery. It’s a not invasive anticancer treatment with almost no side effects and it preserves the eyelid and its adnexas.
Complete remission of a primary unresectable high grade hemangiosarcoma in a dog treated with VAC and antiangiogenic therapy

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Introduction
Hemangiosarcoma (HSA) is a common malignant neoplasm of vascular endothelial cells. Most common affected sites are spleen, right atrium, liver, and skin. Bone, kidney, bladder, muscle and lung can also be primarily affected. Brain is rarely affected of metastatic HSA. Intramuscular HSAs are characterized by an average survival of 172 days after diagnosis and only 25% dogs lived more than 1 year.

Materials and Methods
An 8-year-old female, neutered, boxer was presented to our hospital with an, ill defined, fluctuating mass at the cervical region with deep intramuscular cervical location. Exploratory surgery evidenced a non resectable, deeply infiltrated mass affecting most dorsal cervical muscles. Biopsy report came as highly aggressive grade III intramuscular HSA. Clinical stage was T3N0M0. Two courses of modified VAC protocol followed by antiangiogenic therapy (masitinib, cyclophosphamide, NSAID) were administered as adjuvant treatment.

Results
After 106 days of treatment without evidence of metastatic disease in chest radiographs or abdominal ultrasound, neurological symptoms were reported by owner. MRI confirmed brain lesions highly suggestive of metastatic disease. Dog was euthanized due to rapid deterioration. Necropsy did not show evidence of neoplastic disease at cervical region. Two round, friable, well demarcated, masses were observed in the cerebral hemispheres. Both lesions corresponded histologically with hemangiosarcoma. No other metastatic lesions were observed in the rest of the body.

Conclusions
Consecutive treatment with modified VAC protocol followed by antiangiogenic therapy produced a complete remission of the primary unresectable hemangiosarcoma and avoided tumor dissemination to target organs, without any effect in development of CNS metastasis.
Multiple hair follicular melanocytoma in a dog

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Introduction
Melanocytic neoplasms are commonly diagnosed in dogs. Different classification systems have been used for canine melanocytic proliferations. In accordance with the World Health Organization classification system (Goldschmidt et al., 1998), the term ‘melanocytoma’ is used to encompass all variants of congenital and acquired benign neoplasms arising from melanocytes while ‘melanoma’ is used synonymously with malignant melanoma. Melanocytoma can arise from the melanocytes in the epidermis, dermis, or adnexa. Follicular melanoma (FM) is a rare, recently and scarcely described morphologic variant of melanoma in human beings, and to the author’s knowledge no previous reports of similar lesions in domestic animals have been made.

Materials and Methods
A 6-year-old, male, not spayed, hound dog was presented in November of 2011 with a pigmented plaque of 2.2 x 1.3 cm. The lesion was surgically removed, fixed in 10% buffered formalin, and submitted to histopathological analysis.

Results
Microscopical examination revealed a melanocytoma affecting deepest parts of multiples hair follicles without involvement of the follicular infundibulum nor overlying epidermis. Neoplastic melanocytes were well differentiated with an inconspicuous and clear cytoplasm, well defined nuclei and formed nest of cells that affect the external root sheath of the hair follicles. Mitotic figures were not noted. Immunohistochemical analysis was performed. After two years of follow up the patient remains alive and no recurrence of the primary lesion neither metastasis to lymph node or internal organs have developed.

Conclusions
Based on histological, immunohistochemical and clinical results a diagnosis of multiple hair follicular melanocytoma was made.
Local recurrence control of a large ectopic thyroid adenocarcinoma in a dog using metronomic therapy alone

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Introduction
Ectopic thyroid carcinomas have been described in several studies with two main predilection sites emerging, the cranial mediastinum and the ventral laryngeal area. Despite surgical resection, in many cases these tumors are locally invasive and perioperative morbidity can be high.

Materials and Methods
A 10 years old female spayed boxer was referred to our service with a large, rounded, attached mass in the thoracic inlet. Respiratory distress and dysphagia was present. No other abnormalities were found on physical exam or lab tests. Chest radiographs and abdominal ultrasound showed no evidence of metastases. Mass aspiration produced 160 ml fluid, without evidence of malignant cells. FNA, tru-cut and surgical biopsies were subsequently taken, being all inconclusive. Iodinated contrast injection into the mass did not revealed signs of migration and cavity was well delimited. Surgical resection was performed. Mass was firmly adhered to most cervical structures, but no infiltration was present. Surgical margins were invaded by tumor cells at some areas. No signs of vascular invasion were detected. Histopathologic diagnosis came as high grade thyroid ACA.

Results
Dog was started on metronomic cyclophosphamide (15 mg/kg daily) and meloxycam (0.05 mg/kg eod). Six months later owner decided stop therapy. After three months dog was presented with respiratory distress.Pleural effusion and significant abnormalities on blood work made owner elect euthanasia. Necropsy revealed metastatic disease in several organs. No evidence of recurrence was observed.

Conclusions
Metronomic therapy with cyclophosphamide and meloxycam showed activity over local control of recurrence but no apparent effects on metastasis prevention.
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