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ONCOLOGY

With:

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CANINE MAST CELL TUMORS: MARGINS, MARKERS & PROGNOSTIC FACTORS

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GENERAL INFORMATION
Mast cell tumors (MCT’s) are the most common tumor in the dog and the second most common

tumor in the cat. MCT’s are primarily a disease of older dogs and cats, however, extremely young
dogs and cats have been reported to have MCT’s. Canine breeds reported to be at increased risk for
MCT's are Boxers, Boston terriers, Labrador retrievers, terriers and Beagles. The only feline breed
that has been reported to be at increased risk for MCT’s are Siamese. Most reports show no
significant gender predilection for MCT's in dogs or cats. The etiology of MCT’s is presently
unknown. Many have suspected a viral etiology due to MCT transplantability to susceptible
laboratory dogs (extremely young or immunocompromised) with tumor cells and cell-free extracts.
Recent evidence shows that a significant percentage of dogs with higher-grade MCT’s have genetic
mutations in c-kit (stem cell factor receptor) which may be responsible for the genesis and/or
progression of MCT’s in dogs. Not all dogs with MCT’s have c-kit mutations, suggesting that they are
not the only mechanisms for the development and/or progression of MCT’s.

Eighty-five to ninety percent of dogs and cats with MCT’s have solitary lesions. It is important to
note that not all dogs or cats with multiple MCT’s have metastatic or systemic mastocytosis. Studies
suggest that well-differentiated MCT’s are slow-growing, usually < 3-4 cm in diameter, without
ulceration of overlying skin, variably alopecic and commonly are present for more than 6 months.
In contrast, poorly differentiated MCT’s are rapidly growing, variably sized (but generally large),
with ulceration of the underlying skin and inflammation/edema of surrounding tissues and lastly
rarely are present for more than 2-3 months before presentation. Since most MCT’s are of
moderate-differentiation, signs may be somewhere between these two extremes.

HISTORY & CLINICAL SIGNS
The history and clinical signs of dogs and cats with MCT's can be extremely variable. Most do not
show any clinical signs referable to their MCT, however, some may have signs referable to the
release of heparin, histamine and/or other vasoactive amines. Mechanical manipulation or extreme
changes in temperature can lead to degranulation of MCT’s and subsequent erythema/wheel
formation (Darier’s sign) and gastrointestinal ulceration (anorexia, vomiting, melena, etc.).

DIAGNOSIS & STAGING
Fine needle aspiration and cytology (FNAC) is the mainstay for diagnosis of MCT prior to surgical
removal. Mast cells of MCT’s have a characteristic discrete cell cytological appearance with
eccentrically placed nuclei and abundant red to purple (ie metachromatic) cytoplasmic granules.
Occasional MCT's, predominately undifferentiated MCT’s, do not have the classic metachromatic
cytoplasmic granules and must be diagnosed via other means (histopathology, special stains, etc.).
Once a diagnosis is obtained, staging (looking for disease elsewhere) is routinely recommended,
however, the completeness of staging is presently extremely controversial. After an FNAC diagnosis
of MCT has been made, this author recommends routine staging diagnostics (full physical
examination, bloodwork/urinalysis, FNAC of any local lymph nodes and abdominal ultrasound).
Additional diagnostics such as thoracic radiography and bone marrow aspiration/cytology may be
employed.
The use of buffy coat cytology and liver/spleen FNAC is presently controversial in the routine staging of dogs with MCT and this author does not routinely employ these diagnostics for staging of MCT’s in dogs. Some oncologists have begun to either not routinely utilize bone marrow aspiration & cytology (BMAC) for MCT staging, or have begun to utilize results of CBC/plt to delineate whether or not to perform a BMAC. This is incredibly controversial and results of a recent publication concerning incidence and risk factors of bone marrow infiltration for canine MCT will be presented at the lecture.

**TREATMENT**

Once the diagnosis of MCT has been made with FNAC and/or incisional biopsy and staging has been completed showing no evidence of metastasis to other sites, surgical excision is the preferred choice of therapy. The standard recommendation for complete surgical removal of MCT’s has been three centimeters lateral and 1 fascial plane deep to the MCT. The derivation of this recommendation is unknown. This author still recommends continuing use of 3 cm lateral margins and one fascial plane deep margins whenever possible, but we recently published studies which show that 2 cm lateral and one fascial plane deep margins are sufficient for most grade II MCT. At present, the Seguin et al grade II MCT in dogs paper (2001) has the best information even though the followup time was relatively short (median of only 540 days). Those investigators found a 5% recurrence rate in the face of clean margins, an 11% second primary tumor development rate, and a 5% metastatic rate.

Recent studies in cats with skin/SQ MCT suggest that the vast majority are minimally invasive tumors with low recurrence rates suggesting that as wide and deep surgical margins may not be as necessary in cats as it is in dogs. It can not be over-emphasized as discussed above that cats with dermal MCT should be staged to ensure they do not have a splenic primary MCT that is metastasizing to dermal and/or other sites.

Dogs and cats with incomplete surgical removal of their MCT should undergo re-resection whenever possible. When re-resection is not feasible, external beam radiation therapy has been found to be an excellent post-operative therapeutic modality affording 75-85% control at 4-5 years in dogs with incompletely resected grade II MCT. Recurrence rates for completely resected grade II MCT hover in the 5-20% range in the veterinary oncology literature. Recurrence rates for incompletely resected MCT’s hover in the 25-40% range. At present, we have to recommend additional local therapy for all incompletely resected MCT’s in the face of such low-moderate recurrence rates, and additional recent studies will be discussed at the lecture that may help better predict which cases truly need additional local therapy will be discussed.

The results of a study utilizing radiation therapy for incompletely resected grade III MCT in dogs has been recently published by Hahn et al from Gulf Coast Veterinary Specialists. Thirty-one dogs received 52 Gy of external beam radiation in 18 fractions on a M-W-F basis to the surgical site and draining lymph nodes with no additional therapy (ie no chemotherapy). These investigators found a median survival time of ~ 28 months (range 3-52 months). Only one dog went on to develop systemic MCT metastasis. The results of this trial are controversial within the veterinary oncology community as previous metastatic rates for grade III MCT have been reported to be 55%-96%. At this time, most oncologists are continuing to use chemotherapy in the treatment of grade III MCT; however, results of this study suggest that the more aggressive use of radiation therapy may be beneficial for grade III MCT.

As discussed above, surgery and radiation therapy should be considered the mainstays of therapy for MCT’s. Chemotherapy is a very distant third modality that may be useful for dogs and cats with
systemic or metastatic mast cell tumor. Recent studies suggest that CCNU (lomustine), vinblastine, possibly cyclophosphamide and finally prednisone have limited activity against MCT. The results of studies utilizing chemotherapy will be presented in detail at the lecture.

PROGNOSIS
Histopathologic examination of MCT’s has been found to be an important prognostic indicator by multiple groups. The Patnaik grading scheme (well-differentiated = grade I, moderately-differentiated = grade II and poorly-differentiated = grade III) has shown that 83%, 44% and 6% of dogs with grade I, II and III tumors were alive approximately 4 years after surgery, respectively. This grading scheme has not been found to be of use for cats with MCT. Additional negative prognostic factors include advanced stage, caudal half of body location, high growth rates, aneuploidy and presence of systemic signs. Newly discovered molecularly-based negative prognostic factors include increased AgNOR (silver nucleolar organizing regions) scores, increased PCNA/Ki67 immunohistochemistry (IHC) expression (proliferation markers), increased vascularity and/or mitotic index and increased c-kit IHC expression. The use of panels of the aforementioned prognostic factors is strongly recommended due to their significant predictive ability for both the subsequent development of metastasis as well as subsequent development of recurrence.

REFERENCES:


MAMMARY GLAND TUMORS

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GENERAL INFORMATION
Mammary gland tumors (MGT) are some of the most common tumors seen in veterinary clinical practice. They are the most common tumor seen in the female dog and the second most common tumor of the female cat. The risk of development of a MGT is well known to be increased in dogs that have not undergone ovariohysterectomy at an early age (Schneider et al, JNCI 1969). When compared to an intact female, the risk of development of a MGT in dogs spayed before their first heat, after their first heat and after their second heat is 0.05%, 8% and 26%, respectively. Importantly, this study did not find any reduction in MGT incidence in dogs spayed after their third heat. Early spaying of cats results in only a 50% reduction in MGT incidence, however a recent study suggests the protection is more like four-fold. The culmination of these studies strongly suggests that hormones influence the development of MGT in dogs and cats. In keeping with this tenet, the use of hormones such as synthetic progestins and progesterone has also been found to increase the risk of benign MGT formation in dogs. Though little work has been done to delineate additional risk factors, obesity and home cooked meals has been found to increase the risk of development of MGT in dogs. There are likely genetic influences concerning the etiopathogenesis of MGT in dogs and cats, however, very little research has been done in this area to date.

PATHOLOGY
The pathology of MGT in dogs and cats can be remarkably different. In dogs, approximately 50% are benign and 50% malignant, whereas in cats, 90% or more are malignant. Most MGT in the dog and cat are epithelial in origin (ie adenoma or carcinoma), however, carcinomas and sarcomas are occasionally noted. Mixed mammary tumors are benign tumors of epithelial and mesenchymal origin that are extremely common in the dog. A number of histologic classification schemes have been reported, however, the various histologic categories do not generally make a difference in prognosis clinically. That said, ductular carcinomas and carcino-sarcomas are well known to behave in a more malignant and metastatic fashion. In addition, grade and degree of differentiation have been found to be of prognostic importance in multiple studies. A rarely seen canine MGT subcategory termed Inflammatory Mammary carcinoma (IMC) is diagnosed by histologic and clinical criteria including the presence of a MGT, erythema and/or bruising of the overlying skin, as well as potential blockage of lymphatics in the local area with possible pitting edema of one or both hindlimbs. IMC is an extremely malignant MGT of the dog that routinely has a grave prognosis as most dogs have overt metastasis, or develop overt metastasis, within a very short period of time from presentation.

HISTORY & CLINICAL SIGNS
Dogs have 5 mammary glands, whereas cats have only 4 mammary glands. Interestingly, approximately 60-70% of MGT in dogs and cats occurs in the caudal most 2 mammary glands. In addition, approximately 50% of dogs as well as cats will have a solitary MGT, and the other half will present with multiple MGT. Dogs with multiple MGT on presentation will generally have tumors in a variety of locations, whereas cats with multiple MGT will have multiple tumors within a mammary chain. Though exceptions will always occur, most benign MGT in dogs are small, firm, and well circumscribed lesions that are not adherent to underlying tissues. In cats, because benign tumors are extremely rare, it is important to differentiate the typical malignant MGT from benign mammary fibroadenomatosis (BMF). Cats with BMF are generally younger cats that are intact
females, and pregnancy is commonly noted. Administration of progestins can also be a common historical feature of cats with BMF. While multiple mammary glands in feline BMF are generally swollen and many times painful on palpation, single glands can occasionally be affected in older cats. BMF will generally resolve with cessation of progestin administration and/or spaying. Occasionally, surgical removal of cats with single mammae BMF must be performed if spaying does not resolve the condition.

**DIAGNOSIS & STAGING**

The size of a MGT on presentation can be extremely variable. It is very important that the clinician remember to measure the diameter of the tumor(s) and place this information in the medical record. In addition, a simple description of the MGT is in order, as ulceration and other factors can be prognostic. Approximately 50% of feline MGT are ulcerated at the time of presentation. It is also important to palpate all local lymph nodes, especially the inguinal, axillary and prescapular lymph nodes in dogs and cats suspected of having MGT. If lymphadenopathy is present, a fine needle aspirate (FNA) and cytological examination should be performed; however, palpation is a relatively insensitive measure of lymph node metastasis and considerations should be made for LN FNA/cytology independent of whether the lymph node is palpable or not. In addition, any other abnormalities on physical examination (PE), especially lameness or other masses should be fully worked up to ensure they are not metastases of the MGT. In addition to that listed above, a thorough PE, a CBC/platelet count, small animal diagnostic profile and UA (retroviral and thyroid testing as well in cats) should be performed. Similarly, 3-view chest radiographs should be also be performed. An abdominal ultrasound should also be strongly considered, especially for dogs with caudal MGT or any cat with MGT, since ~ 25% of cats can have abdominal metastases of their MGT. In addition, to further delineate the problem at the suspected MGT site, an FNA/cytology of the mass should be performed to determine if the mass is a MGT, some other type of tumor or a non-neoplastic process. This is extremely controversial; however, this author feels strongly that one should start with an FNA/cytology. Though the cytological examination may have difficulty discerning benign from malignant, the result will tell the clinician with high probability if some other process is ongoing and then an appropriate workup/staging can then be performed. For example, a mast cell tumor that just happens to be in the general mammary area will be staged and treated potentially very differently than a mammary gland tumor.

**TREATMENT**

Surgery continues to be the gold standard therapy for dogs and cats diagnosed with MGT. The recommended surgical procedure varies between dogs and cats due to: 1) the chance of a malignancy across species (50% in dogs, > 90% in cats), and 2) the chance for recurrence with minimal excision (low in dogs, high in cats). Therefore, minimal excision via lumpectomy or mammaectomy is recommended for dogs with solitary MGT, whereas radical mastectomy is recommended for cats with MGT. A chain mastectomy should be considered in dogs only when there are multiple lesions in a chain, as the chain mastectomy will be procedurally easier than multiple lumpectomy or mammaectomy. Considerations should be made in dogs and cats for axillary and inguinal LN dissection and histopathological examination with cranial and caudal gland MGT, respectively. In addition, considerations should be made for staged bilateral radical mastectomies in cats, especially those with better prognoses, as the chance for additional primary MGT that are malignant are extremely high. This is extremely controversial, but no different than the recommendations for prophylactic bilateral radical mastectomy in women with mutant breast cancer genes as the surgical procedure allows for early removal of multiple incipient disease areas for further cancer and potential metastasis can occur.
Additional therapies for dogs and cats with MGT exist, however, the clinical usefulness of these therapies is presently limited due to the paucity of studies presently available. For example, the use of chemotherapy as an adjuvant treatment of dogs with MGT is presently largely unexplored. Similarly, relatively few studies have explored the use of chemotherapy in cats with unresectable or metastatic MGT and no published studies are available to date concerning the adjuvant use of chemotherapy in cats with micro-metastatic MGT. A recently published study by Novosad and this author in a large series of cats with malignant MGT treated with 5 doses of doxorubicin after surgery found an approximate doubling of survival compared to historical controls published by MacEwen et al in 1984. The use of chemotherapy as an adjuvant treatment in dogs with high-risk MGT and cats with ≥ Stage II MGT should be considered, however, the therapeutic efficacy of this approach remains to be determined. Palliative radiation therapy (large dose per fraction & relatively few fractions) can be considered for IMC and tumors that are extremely large and causing significant quality of life concerns. Biologic response modifiers have to date not been found to efficacious against MGT.

The effect of ovariohysterectomy (OHE) at the time of primary tumor removal is presently extremely controversial. Three previous studies have found no effect; however, recent studies by Sorenmo et al and Chang et al have found an increase in survival for dogs that underwent OHE at the time of their primary MGT removal or ≤ 2 years of the time of their primary MGT removal. At the very least, an OHE should be strongly considered in conjunction with the MGT resection to reduce the chance of subsequent pyometra; however, the effect of OHE on subsequent MGT and metastasis desperately needs further definitive study. The use of estrogen receptor blockers such as tamoxifen is also extremely controversial as separate studies show a pronounced anti-tumor effect and no effect, respectively. Tamoxifen usage is contraindicated in intact female dogs and cats due to pyometra, and the use of tamoxifen in spayed dogs and cats can result in stump pyometra, urinary tract infections, nesting behavior, etc. Since studies do not presently show documented clinical benefit in dogs and cats, their use on a routine basis is presently not recommended by this author.

**PROGNOSIS**

The understanding of prognostic factors for dogs and cats with MGT is of paramount importance. These factors will help the clinician educate the client about their pet's prognosis and then direct questions and decisions about further therapy. The following factors are associated with a poor prognosis in dogs with MGT: 1) lymph node metastasis or other metastasis, 2) IMC, 3) carcinosarcoma or ductular carcinoma, 4) high grade tumor and/or vasculo-lymphatic invasion, 5) ≥ 3 cm. primary tumor, 6) invasive and/or fixed to underlying tissue, 7) high AgNOR count, 8) estrogen receptor negative, 9) ulceration, 10) lack of lymphoid cell reactivity, 11) increased microvessel density, 12) increased proliferation index scores (AgNOR and Ki-67) and 13) increased VEGF expression. The following factors are associated with a poor prognosis in cats with MGT: 1) lymph node metastasis or other metastasis, 2) tumor size and stage (≥ 2-3 cm in diameter do worst), 3) higher grade tumors (poorly differentiated, increased mitotic figures, increased necrosis, etc.), 4) conservative surgery (vs. preferred radical mastectomy), 5) breed (Siamese & tricolor's do worse), 6) increasing age, 7) higher AgNOR counts, and 8) vasculo-lymphatic invasion. When dogs with MGT have poor prognostic factors as discussed above, this suggests they are at increased risk for metastasis and therefore adjuvant chemotherapy should be discussed with the client. While it is presently unknown which cats should have adjuvant chemotherapy, this author believes it should be strongly considered in most cats with malignant MGT since this is such an aggressive and metastatic malignancy.

MGT's are extremely common tumors in veterinary practice. The biologic heterogeneity seen with these tumors can be extreme; this underscores the need to have a greater understanding of the
biology and prognostic factors of this disease. Surgery can be curative for approximately 75% of dogs with MGT, whereas cats with MGT generally have a poor prognosis. That said, the astute clinician when armed with an understanding of the biology and prognostic factors of dogs and cats with MGT, will be able to determine in advance the occasional situations where dogs have a poor prognosis MGT and when cats have a decent prognosis MGT and then counsel that client for the best therapies in each situation.

SELECTED REFERENCES:

This discussion will review what I feel to be the top 10 clinically relevant advances in veterinary oncology over the last ~ 10 years. I will post the abstracts from these publications and then summarize them in the lecture. It is important to point out that major advances in surgical and radiation oncology have occurred over the last 30 years (e.g. hemipelvectomy, limb-sparing, nasal planectomy, scapulectomy, etc.) but they will not be discussed here.

1. Six month chemo for lymphoma.\(^{(1)}\) The purpose of this study was to compare a maintenance-free chemotherapy protocol based on CHOP (H from hydroxydaunorubicin = doxorubicin, O from Oncovin = vincristine) to a similar protocol with a maintenance phase for the treatment of canine lymphoma. Fifty-three dogs with multicentric lymphoma were treated with a 6-month modified version of the University of Wisconsin (UW)-Madison chemotherapy protocol (UW-25). Disease-free interval (DFI) and survival were compared to a historical control group of 55 dogs treated with a similar protocol with a prolonged maintenance phase. Remission rate for the study dogs was 94.2% (complete remission = 92.3%, partial remission = 1.9%). DFI and survival between the 2 groups did not differ significantly, with median DFI and survival of the study dogs equal to 282 and 397 days compared to 220 and 303 days for the control dogs (\(P = .2835\) and \(.3365\), respectively). Univariate analysis identified substage b (\(P = .0087\)), German Shepherd breed (\(P = .0199\)), and body weight > 18 kg (\(P = .0016\)) as significant for worse survival. Longer survival was associated with thrombocytopenia (\(P = .0436\)). Multivariate analysis revealed that substage (\(P = .0388\)) and weight (\(P = .0125\)) retained significance for DFI, whereas substage (\(P = .0093\)), thrombocytopenia (\(P = .0150\)), and weight (\(P = .0050\)) retained significance for survival. Overall, the protocol was well tolerated by the dogs, with 41.5% (22/53) requiring a treatment delay or dose modification, but only 9.4% (5/53) needing hospitalization. The 6-month chemotherapy protocol based on CHOP with no maintenance phase provides similar DFI and survival times when compared to a similar protocol with a prolonged maintenance phase.

2. FNA of non-palpable LN's.\(^{(2)}\) OBJECTIVE: To determine sensitivity and specificity of physical examination, fine-needle aspiration, and needle core biopsy of the regional lymph nodes for evidence of metastasis in dogs and cats with solid tumors. DESIGN: Case series. ANIMALS: 37 dogs and 7 cats. PROCEDURE: Regional lymph nodes were evaluated by means of physical examination (palpation), fine-needle aspiration, and needle core biopsy. Results were compared with results of histologic examination of the entire lymph node, the current standard. RESULTS: Tumors included 18 sarcomas, 16 carcinomas, 7 mast cell tumors, and 3 other tumors. Carcinomas were more likely to have metastasized to the regional lymph node (7/16 animals) than were sarcomas (2/18). Sensitivity and specificity of physical examination were 60 and 72%, respectively. Sensitivity and specificity of cytologic examination of fine-needle aspirates were 100 and 96%, respectively. Sensitivity and specificity of histologic examination of needle core biopsy specimens were 64 and 96%, respectively. CONCLUSIONS AND CLINICAL RELEVANCE: Results suggested that fine-needle aspiration may be a sensitive and specific method of evaluating the regional lymph nodes in dogs and cats with solid tumors, because results correlated well with results of histologic examination of the entire lymph node. Physical examination alone was not a reliable method and should not be used to decide whether to aspirate or biopsy the regional lymph nodes.
3. Use of CT for delineation of metastases.
A. Imaging studies in people indicate that x-ray computed tomography (CT) is a more sensitive technique than thoracic radiography for the detection of pulmonary metastatic neoplasia. Systematic studies comparing CT and thoracic radiographic techniques in veterinary patients have not been performed. The present retrospective study was designed to directly compare the efficacy of these 2 techniques in detecting pulmonary nodules in dogs. Eighteen dogs with histologically confirmed pulmonary metastatic neoplasia had contemporaneous thoracic radiographs and pulmonary CT scans compared. Quantitative analyses included estimation of pulmonary nodule size, number, and lobar distribution on thoracic radiographs and CT images. Only 9% of CT-detected pulmonary nodules were identified on thoracic radiographs (P < .003). The lower size threshold was approximately 1 mm to detect pulmonary nodules on CT images and 7-9 mm to reliably detect nodules on radiographs (P < .0001). Additionally, pulmonary nodules were detected in a significantly greater number of lung lobes using CT as compared with thoracic radiographs (P < .0001). These data indicate that CT is significantly more sensitive than thoracic radiography for detecting soft-tissue nodules in dogs. As such, thoracic CT should be considered in any patient with neoplasia that has potential for pulmonary metastasis to more reliably stage the disease, particularly when accurate characterization of the extent and distribution of pulmonary metastatic disease affects therapeutic planning.(3)

B. OBJECTIVE: To compare results of computed tomography (CT) and radiography with histopathologic findings in tracheobronchial lymph nodes (TBLNs) in dogs with primary lung tumors. DESIGN: Retrospective case series. ANIMALS: 14 client-owned dogs. PROCEDURES: Criteria for inclusion were diagnosis of primary lung tumor, use of thoracic radiography and CT, and histologic confirmation of TBLN status. Medical records were reviewed for signalment; history; and physical examination, clinicopathologic, radiographic, CT, surgical, and histopathologic findings. RESULTS: Tracheobronchial lymphadenopathy was not identified via radiography in any dogs. Tracheobronchial lymphadenopathy was diagnosed in 5 dogs via CT. Six dogs had histologic confirmation of metastasis to TBLNs. Radiographic diagnosis yielded 6 false-negative and no false-positive results for tracheobronchial lymphadenopathy. Computed tomography yielded 1 false-negative and no false-positive results. Sensitivity of CT for correctly assessing TBLN status was 83%, and specificity was 100%. Positive predictive value was 100%, and negative predictive value was 89%. Dogs with lymphadenopathy via CT, histologic confirmation of TBLN metastasis, or primary tumors with a histologic grade > 1 had significantly shorter survival times than their counterparts. CONCLUSIONS AND CLINICAL RELEVANCE: Results of CT evaluation of TBLN status were in agreement with histopathologic findings and more accurate than use of thoracic radiography for evaluating TBLNs in dogs with primary lung tumors. Computed tomography imaging should be considered as part of the staging process to more accurately assess the TBLNs in dogs with primary lung tumors.(4)

A. Chemotherapy-induced nausea and vomiting (CINV) is a common side-effect of cisplatin therapy. Maropitant (Cerenia TM ), a novel neurokinin-1 receptor antagonist, was evaluated for prevention and treatment of cisplatin-induced emesis in tumour-bearing dogs. Dogs (n = 122) were randomly allocated to three treatment groups: T01, placebo before and after cisplatin; T02, placebo before and maropitant after cisplatin; or T03, maropitant before and placebo after cisplatin. Maropitant treatment (T02) following a cisplatin-induced-emetic event resulted in significantly fewer subsequent emetic events (P = 0.0005) than in placebo-treated dogs (T01). In placebo-treated (T01) dogs, 56.4% were withdrawn from the study because of treatment failure compared with 5.3% in group T02. When maropitant was administered prior to cisplatin treatment (T03) in a prevention regime, 94.9% did not vomit compared with only 4.9% of placebo-treated dogs, and
significantly fewer emetic events (P < 0.0001) were observed in those dogs that did vomit. In summary, maropitant was safe and highly effective in reducing or completely preventing cisplatin-induced emesis.(5)

B. Maropitant (Cerenia), a selective neurokinin(1) receptor antagonist, was evaluated for safety and efficacy in treatment and prevention of acute vomiting due to various etiologies in dogs in a randomized clinical trial. Two-hundred seventy-eight dogs were enrolled from 29 veterinary hospitals. Two-hundred fifty-two were evaluable for efficacy, while 275 were evaluable for safety. A randomized block design was utilized (three maropitant- and one placebo-treated dog per block). Initial treatment was maropitant at 1 mg/kg body weight (0.45 mg/lb) or an equivalent volume of saline (placebo) administered subcutaneously. On the subsequent 1 to 4 days, maropitant or placebo (dependent on allocation) was administered subcutaneously or orally at approximate 24-h intervals as needed. Oral doses were administered as maropitant tablets using unit dosing to deliver a minimum dose of 2 mg/kg body weight (0.9 mg/lb) or equivalent numbers of similar placebo tablets. Dogs and housing were observed twice daily for evidence of vomiting. Emesis was significantly (P <or= 0.0012) reduced in maropitant-treated dogs as 50% (32/64) of placebo-treated dogs continued to vomit compared to only 21.8% (41/188) of maropitant-treated dogs. Post-treatment clinical signs were consistent with clinical diagnoses and judged not to be treatment related. In this clinical trial, maropitant was safe and effective in reducing emesis due to various etiologies in dogs.(6;7)

5. Palladia.(8)
A. The purpose of this study was to determine the objective response rate (ORR) following treatment of canine mast cell tumors (MCT) with toceranib phosphate (Palladia, SU11654), a kinase inhibitor with both antitumor and antiangiogenic activity through inhibition of KIT, vascular endothelial growth factor receptor 2, and PDGFRalpha. Secondary objectives were to determine biological response rate, time to tumor progression, duration of objective response, health-related quality of life, and safety of Palladia. EXPERIMENTAL DESIGN: Dogs were randomized to receive oral Palladia 3.25 mg/kg or placebo every other day for 6 weeks in the blinded phase. Thereafter, eligible dogs received open-label Palladia. RESULTS: The blinded phase ORR in Palladia-treated dogs (n = 86) was 37.2% (7 complete response, 25 partial response) versus 7.9% (5 partial response) in placebo-treated dogs (n = 63; P = 0.0004). Of 58 dogs that received Palladia following placebo-escape, 41.4% (8 complete response, 16 partial response) experienced objective response. The ORR for all 145 dogs receiving Palladia was 42.8% (21 complete response, 41 partial response); among the 62 responders, the median duration of objective response and time to tumor progression was 12.0 weeks and 18.1 weeks, respectively. Palladia-treated responders scored higher on health-related quality of life versus Palladia-treated nonresponders (P = 0.030). There was no significant difference in the number of dogs with grade 3/4 (of 4) adverse events; adverse events were generally manageable with dose modification and/or supportive care. CONCLUSIONS: Palladia has biological activity against canine MCTs and can be administered on a continuous schedule without need for routine planned treatment breaks. This clinical trial further shows that spontaneous tumors in dogs are good models to evaluate therapeutic index of targeted therapeutics in a clinical setting.

6. Melanoma Vaccine.(9-13)
A. Canine malignant melanoma (CMM) is an aggressive neoplasm treated with surgery and/or fractionated RT; however, metastatic disease is common and chemoresistant. Preclinical and clinical studies by our laboratory and others have shown that xenogeneic DNA vaccination with tyrosinase family members can produce immune responses resulting in tumor rejection or protection and prolongation of survival. These studies provided the impetus for development of a
xenogeneic DNA vaccine program in CMM. MATERIALS AND METHODS: Cohorts of three dogs each received increasing doses of xenogeneic plasmid DNA encoding either human tyrosinase (huTyr; 100/500/1500 mcg), murine GP75 (muGP75; 100/500/1500 mcg), murine tyrosinase (muTyr; 5 dogs each at 100/500 mcg), muTyr+/−HuGM-CSF (9 dogs at 50 mcg muTyr, 3 dogs each at 100/400/800 mcg HuGM-CSF, or 3 dogs each at 50 mcg muTyr with 100/400/800 mcg HuGM-CSF), or 50 mcg MuTyr intramuscularly biweekly for a total of four vaccinations. RESULTS: The Kaplan-Meier median survival time (KM MST) for all stage II-IV dogs treated with huTyr, muGP75 and muTyr are 389, 153 and 224 days, respectively. Preliminarily, the KM MST for stage II-IV dogs treated with 50 mcg MuTyr, 100/400/800 mcg HuGM-CSF or combination MuTyr/HuGM-CSF are 242, 148 and >402 (median not reached) days, respectively. Thirty-three stage II-III dogs with loco-regionally controlled CMM across the xenogeneic vaccine studies have a KM MST of 569 days. Minimal to mild pain was noted on vaccination and one dog experienced vitiligo. We have recently investigated antigen specific antibody and T-cell responses in dogs vaccinated with HuTyr.

CONCLUSIONS: The results of these trials demonstrate that xenogeneic DNA vaccination in CMM: (1) is safe, (2) leads to the development of anti-tyrosinase antibodies, (3) is potentially therapeutic, and (4) is an attractive candidate for further evaluation in an adjuvant, minimal residual disease Phase II setting for CMM.

7. Diagnostic Imaging Advances. To be reviewed at the lecture with special emphasis on importance to feline vaccine-associated sarcoma.

8. Do we really need Elspar for Lymphoma? A. The purpose of this study was to evaluate response rates, 1st remission duration (FRD), and toxicity in dogs with previously untreated lymphoma receiving an identical CHOP-based combination chemotherapy protocol with or without L-asparaginase (LASP). One hundred fifteen dogs with lymphoma were scheduled to receive an identical CHOP-based chemotherapy protocol that included L-ASP. However, because of manufacturer-imposed random rationing, 31 dogs did not receive L-ASP as scheduled. The 2 treatment groups were statistically similar with respect to signalment and presence of historical negative prognostic factors. No difference was observed in the median FRD whether dogs did or did not receive L-ASP (206 versus 217 days, respectively; P = .67). No difference was observed in the median overall survival times between dogs receiving or not receiving L-ASP (310 versus 308 days, respectively; P = .84). No statistical difference was observed with respect to overall response rate between dogs that did or did not receive L-ASP (89.3% versus 87.1%, respectively; P = .75). Complete response rates between the groups also were no different (83.3% and 77.4% for L-ASP and non-L-ASP groups, respectively; P = .59). Prevalence of toxicity (neutropenia, diarrhea, or vomiting) and treatment delays (P = .80) also were similar between groups. The results of this study suggest that exclusion of L-ASP in this multidrug protocol does not significantly impact outcome. Therefore, it may be more appropriate to reserve the use of L-ASP for treating relapse in dogs with lymphoma that have failed induction therapy.(14)

B. Combination chemotherapy is superior to single-agent chemotherapy for treating canine lymphoma, but the effect of each drug on efficacy remains unknown. By comparing 34 dogs treated with a modified cyclophosphamide, vincristine, prednisone (COP) chemotherapy protocol and 42 dogs given asparaginase in the induction phase of the same protocol, the effect of asparaginase on the chemotherapeutic protocol was determined. Both groups were compared based on clinical response at 2 weeks and 6 weeks, and on the progression-free interval. Asparaginase did not significantly increase the likelihood of a clinical remission or prolong the initial progression-free interval in the dogs studied.(15)
9. **Zinecard for the attenuation of side effects from adriamycin/doxorubicin extravasation.** To be reviewed at the lecture with special emphasis on practical use of this extremely new medication.(16;17)

10. **MDR testing.** To be reviewed at the lecture with special emphasis on chemotherapy sensitivity and importance with drugs outside of oncology.(18-20)

**REFERENCES:**


LYMPHOMA: ANYTHING NEW?

Philip J. Bergman DVM, MS, PhD, DACVIM (Oncology)
Director, Clinical Studies – VCA Antech
Medical Director, Katonah-Bedford Veterinary Center

GENERAL INFORMATION
Lymphoma (LSA) is the most common tumor of the cat and represents approximately 80-90% of hematopoietic tumors in cats. LSA is the third most common tumor in the dog with an estimated annual incidence of 13-24/100,000 dogs at risk. The mean age of cats diagnosed with LSA over 10-15 years ago was 2-5 years of age, however, recent reports suggest the mean age of cats diagnosed with LSA is now 8-12 years. The mean age of dogs afflicted with LSA remains stable at 6-9 years of age, however, the range of age in dogs can be as short as weeks to months. The most common site of LSA diagnosis in cats from over 10-15 years ago was mediastinal and/or multicentric, whereas recent reports suggest the most common site presently is alimentary. Why has there been such a significant change over the years??

Much of this sea-change in age of onset and location for cats with LSA can be attributed to changes in feline leukemia virus (FeLV). FeLV was the most common cause of hematopoietic tumors in cats, and these cats generally had T-cell mediastinal LSA. B cell alimentary LSA in cats is usually seen in older FeLV negative cats, and this is by far the most common presentation for cats presently. Some oncologists believe that all cats with LSA are FeLV positive. This author disagrees with this statement, as specific viruses have never been found to be responsible for all types of LSA in other species, and evidence for strong associations with certain herbicides (e.g. 2,4-D) continues to accumulate in people. Some oncologists believe that the rise in alimentary LSA seen recently is due to a decreased incidence of FeLV with a concomitant increase in food-related carcinogens, though no scientific evidence for the latter is available.

LYMPHOMA CATEGORIZATION & CLASSIFICATION
Dogs & cats with LSA are generally categorized based on anatomic and histologic classifications. The five major anatomical sites are alimentary, mediastinal, multicentric, leukemia and extra-nodal (CNS, cutaneous, other). Though there are a number of histologic classification systems available, the NIH Working Formulation has been the system most widely adopted by histopathologists. This system generally suggests that approximately 10%, 30% and 60% of dogs and cats with LSA have low, intermediate and high-grade tumors, respectively.

HISTORY & CLINICAL SIGNS
The history and clinical signs of dogs & cats with LSA are extremely variable and dependent on the extent of disease and anatomic location. For example, cats with alimentary LSA usually present for anorexia/weight loss, vomiting, diarrhea and an abdominal mass, whereas cats with mediastinal LSA usually present for tachypnea, dyspnea and vomiting/regurgitation. Many dogs with multicentric LSA present for abnormal lumps being found by the owner or groomer, or on routine physical examination by a veterinarian.

DIAGNOSIS
The diagnostic evaluation of dogs & cats with a suspicious diagnosis of LSA should include a full physical examination, bloodwork (CBC/platelet/biochemistry profile), retroviral testing in cats (FeLV/FIV) and urinalysis. Additional staging diagnostics may include abdominal radiography and/or ultrasonography, chest radiography and bone marrow aspiration/cytology. Additional tests may be necessary depending on the anatomic location of the LSA (e.g. mediastinal aspirate for...
mediastinal mass). Caution is noted for NOT making the diagnosis of multicentric LSA off of fine needle aspiration and cytology specifically in cats due to the common syndrome of non-neoplastic retroviral-associated lymphadenopathy. Similarly, the diagnosis of LSA should not be made cytologically with fine needle aspirates of the mandibular lymph nodes in dogs as these lymph nodes are responsible for drainage of the oral cavity, and may have focal areas of hyperplasia that could cytologically mimic LSA.

**TREATMENT**
The last 20 years have shown significant advancements in the treatment of canine LSA, however, such advances have not been made in the treatment of feline LSA. The chemotherapeutic agents and protocols used in dogs are the same ones used in cats. These agents include cyclophosphamide, vincristine, prednisone, doxorubicin, methotrexate and L-asparaginase. The same approximate dosages for the above drugs can be used in dogs as well as cats except for doxorubicin. When cats are given doxorubicin at the originally described 30 mg/m² dose, they may experience significant toxicity including myelosuppression, vomiting, diarrhea, and hepato-/nephrotoxicity. When cats are given doxorubicin at 1 mg/kg, the toxicity is quite manageable and typically self-limiting. In addition, the induction of adriamycin-associated cardiomyopathy that can be seen in dogs and humans is rarely if ever seen in cats.

Combination chemotherapy protocols generally induce a complete remission in 70-85% of dogs and 50-60% of cats with LSA. Similarly, the median remission time for dogs is generally 6-11 months, whereas in cats it is 4-5 months. The median survival time of dogs on multi-agent chemotherapy protocols is 12-26 months, whereas in cats it is only 5-7 months. That said, the range of remission times and survival times in cats can be extremely wide, ranging from weeks to years. It is also important to note that it is extremely difficult to recommend precise treatments for the wide variety of clinical types of LSA seen in dogs and cats. Though studies have not specifically addressed this, this author believes that cats generally tolerate chemotherapy much better than dogs do. Other treatment modalities such as radiation therapy can be utilized in dogs and cats with mediastinal, nasal and CNS LSA, whereas surgery may be useful for dogs and cats with truly extra-nodal non-metastatic LSA (e.g. single small mycosis fungoides or epitheliotropic LSA lesion). A significant amount of time during the presentation will be allotted to discuss options and case scenarios for rescue chemotherapy with lymphoma.

**PROGNOSIS**
The prognosis for dogs and cats with LSA is extremely variable. A large number of prognostic factors have been identified in the dog and these will be presented and ranked as much as possible at the oral discussion. The duration and response to therapy will depend on stage, location and FeLV status. Recent studies suggest that the most important negative prognostic factors are lack of response to therapy, FeLV+, whether the cat is sick or not, advanced stage and lack of doxorubicin in the chemotherapy protocol. This author and others have noted extremely variable remission and survival times for cats with alimentary LSA treated with a wide variety of chemotherapy protocols, ranging from leukeran and prednisone (a la Fondacaro) to typical aggressive multi-agent chemotherapy protocols. This author is presently investigating: 1) the potential for multiple subclassifications of alimentary LSA in cats with hopeful prognostic and therapeutic significance, and 2) the use of immunohistochemical-based prognostic panels utilizing known prognostic factors (AgNOR, immunophenotype, proliferation markers, drug resistance proteins, etc.).
## CANINE LSA PROGNOSTIC FACTORS:

<table>
<thead>
<tr>
<th>Strong</th>
<th>Medium</th>
<th>New</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substage</td>
<td>Stage</td>
<td>Proliferation Markers</td>
</tr>
<tr>
<td>Grade</td>
<td>Hypercalcemia</td>
<td>P-Glycoprotein</td>
</tr>
<tr>
<td>Immunophenotype</td>
<td>Gender</td>
<td>Pulmonary</td>
</tr>
<tr>
<td>Location</td>
<td>Weight</td>
<td>Steroid Use</td>
</tr>
<tr>
<td>Response to Rx</td>
<td>Hypoalbuminemia</td>
<td>Apoptotic Markers</td>
</tr>
</tbody>
</table>

## CANINE LYMPHOMA CHEMOTHERAPY & RESPONSE:

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Remission %</th>
<th>Median Rem. (Mos.)</th>
<th>Mortality from Therapy</th>
<th>Median Surv (Mos.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0%</td>
<td>0</td>
<td>None</td>
<td>1-2</td>
</tr>
<tr>
<td>Pred only</td>
<td>33%</td>
<td>1</td>
<td>Very Low</td>
<td>2</td>
</tr>
<tr>
<td>COP or Adria</td>
<td>60-77%</td>
<td>4-6</td>
<td>Low</td>
<td>6-8</td>
</tr>
<tr>
<td>CVT-X or CHOP</td>
<td>80-82%</td>
<td>5-8</td>
<td>Low</td>
<td>8-11</td>
</tr>
<tr>
<td>ACOPA I &amp; II</td>
<td>75-88%</td>
<td>8-9</td>
<td>Medium</td>
<td>N/A</td>
</tr>
<tr>
<td>VELCAP-S</td>
<td>68-87%</td>
<td>9</td>
<td>Medium</td>
<td>N/A</td>
</tr>
<tr>
<td>VELCAP-L</td>
<td>69%</td>
<td>13</td>
<td>Medium</td>
<td>N/A</td>
</tr>
<tr>
<td>UW-Madison (2 yr.)</td>
<td>82-85%</td>
<td>8.5</td>
<td>Low</td>
<td>11-12</td>
</tr>
<tr>
<td>UW-Madison (6 mo.)</td>
<td>91-94%</td>
<td>9</td>
<td>Low</td>
<td>13</td>
</tr>
</tbody>
</table>
REFERENCES:

INTRODUCTION

Canine malignant melanoma (CMM) of the oral cavity, nail bed, foot pad and mucocutaneous junction is a spontaneously occurring, highly aggressive and frequently metastatic neoplasm. CMM is a relatively common diagnosis representing ~4% of all canine tumors and it is the most common oral tumor in the dog. CMM and advanced human melanoma (HM) are diseases that are initially treated with aggressive local therapies including surgery and/or fractionated radiation therapy; however, systemic metastatic disease is a common sequela. Based on these similarities, CMM appears to be a good clinical model for evaluating new treatments for advanced HM.

Canine patients with advanced disease (WHO stage II, III or IV) have a reported median survival time of 1-5 months with standardized therapies. A combination of hypo-fractionated radiation therapy and chemotherapy have a reported median survival time of one year in stage I oral CMM. Human patients with deep AJCC stage II or stage III disease (locally advanced or regional lymph node involvement) have at least a 50% chance of recurrence after surgical resection; patients with stage IV melanoma (distant metastases) have a median survival of less than ten months and most of these patients eventually die of melanoma. Standard systemic therapy is dacarbazine chemotherapy in HM, and carboplatin chemotherapy in CMM. Unfortunately, response rates to chemotherapy in humans or dogs with advanced melanoma range from 8-28% with little evidence that treatment improves survival. It is easily evident that new approaches to this disease are desperately needed and multiple methodologies have been reported to date.

Active immunotherapy in the form of vaccines represents one potential therapeutic strategy for melanoma. The advent of DNA vaccination circumvents some of the previously encountered hurdles in vaccine development. DNA is relatively inexpensive and simple to purify in large quantity. The antigen of interest is cloned into a bacterial expression plasmid with a constitutively active promoter. The plasmid is introduced into the skin or muscle with an intradermal or intramuscular injection. Once in the skin or muscle, professional antigen presenting cells, particularly dendritic cells, are able to present the transcribed and translated antigen in the proper context of major histocompatibility complex and costimulatory molecules. The bacterial and plasmid DNA itself contains immunostimulatory sequences that may act as a potent immunological adjuvant in the immune response. In clinical trials for infectious disease, DNA immunization has been shown to be safe and effective in inducing immune responses to malaria and human immunodeficiency virus. Although DNA vaccines have induced immune responses to viral proteins, vaccinating against tissue specific self-proteins on cancer cells is clearly a more difficult problem. One way to induce immunity against a tissue specific differentiation antigen on cancer cells is to vaccinate with xenogeneic antigen or DNA that is homologous to the cancer antigen. It has been shown that vaccination of mice with DNA encoding cancer differentiation antigens is ineffective when self-DNA is used, but tumor immunity can be induced by orthologous DNA from another species.

We have chosen to target defined melanoma differentiation antigens of the tyrosinase family. Tyrosinase is a melanosomal glycoprotein, essential in melanin synthesis. The full length human tyrosinase gene was shown to consist of five exons and was localized to chromosome 11q14-q21. Immunization with xenogeneic human DNA encoding tyrosinase family proteins induced antibodies and cytotoxic T cells against syngeneic B16 melanoma cells in C57BL/6 mice, but immunization with mouse tyrosinase-related DNA did not induce detectable immunity. In
particular, xenogeneic DNA vaccination induced tumor protection from syngeneic melanoma challenge and autoimmune hypopigmentation. Thus, xenogeneic DNA vaccination could break tolerance against a self tumor differentiation antigen, inducing antibody, T-cell and anti-tumor responses.

**EXPERIMENTAL DESIGN**

From April 2000 to June 2007, approximately 500 dogs with previously histologically confirmed spontaneous malignant melanoma were treated with xenogeneic DNA vaccinations. Pre-trial evaluation included complete physical examination, a complete blood count and platelet count, serum chemistry profile, urinalysis, lactate dehydrogenase, anti-nuclear antibody, and three-dimensional measurements of the primary tumor if present (or maximal tumor size from medical records if patient was treated prior to pre-trial considerations). For evaluation of metastatic disease, 3-view radiographs of the thorax were obtained and regional lymph nodes were evaluated with fine needle aspiration/cytology and/or biopsy/histopathology. All dogs were clinically staged according to the WHO staging system of stage I (tumor < 2 cm diameter), II (tumors 2-4 cm diameter, negative nodes), stage III (tumor > 4 cm and/or positive nodes) or stage IV (distant metastatic disease). The numbers of previous treatments with surgery, radiation and/or chemotherapy were recorded. Dogs with WHO stage II, III or IV histologically confirmed malignant melanoma were allowed entrance onto the various studies due to the lack of effective available systemic treatments. Due to a strong safety profile, dogs with stage I melanoma were allowed inclusion from 2005 on. Additional entry criteria included: an estimated life expectancy of 6 weeks or more, free of clinically detectable brain metastases, no previous therapy (surgery, radiation and/or chemotherapy) for at least 3 weeks and no serious intercurrent medical illnesses. Written consent for entry onto this trial was obtained from each dog's owner prior to entry onto the study; this consent included request for necropsy upon death due to any reason. These studies were performed under Animal Medical Center IRB approval.

Cohorts of three dogs each received increasing doses of xenogeneic plasmid DNA encoding either human tyrosinase (huTyr; 100, 500 or 1500mcg), murine GP75 (muGP75; 100, 500 or 1500mcg), murine tyrosinase (muTyr; 5 dogs at 100mcg or 500mcg each), or muTyr + HuGM-CSF (9 dogs at 50mcg muTyr, 3 dogs each at 100, 400 or 800mcg HuGM-CSF, or 3 dogs each at 50mcg muTyr with 100, 400 or 800mcg HuGM-CSF) intramuscularly biweekly for a total of 4 vaccinations in the left caudal thigh with the Biojector 2000 jet delivery device with #3 (intramuscular) Bioject syringes. The Biojector2000 is a carbon dioxide powered jet delivery device which is FDA approved for administration of intramuscular injections and has been used in DNA vaccine clinical investigations. Subjective pain level responses and post-vaccinal presence of a wheal or other reaction were assessed and recorded by the veterinarian administering the DNA vaccination. The dogs did not receive any concomitant treatments during the course of vaccination.

**RESULTS**

The signalments of dogs on this study have been similar to those in previously reported CMM studies. No toxicity was seen in any dogs receiving the aforementioned vaccines with the exception of minimal to mild pain responses at vaccination, one muGP75 dog experienced mild aural depigmentation, and one muTyr dog has experienced moderate foot pad vitiligo. Dogs with stage II-III loco-regionally controlled CMM across the xenogeneic vaccine studies have a Kaplan-Meier (KM) median survival time (MST) of > 2 years (median not yet reached). The KM MST for all stage II-IV dogs treated with huTyr, muGP75 and muTyr are 389, 153 and 224 days, respectively. The KM MST for stage II-IV dogs treated with 50mcg MuTyr, 100/400/800mcg HuGM-CSF or combination MuTyr/HuGM-CSF are 242, 148 and > 900 (median not reached, 6/9 dogs still alive) days, respectively. For dogs on the Phase Ib MuTyr/HuGM-CSF/Combination trial, significant differences in MST were noted across pre-vaccination stage (stage IV MST = 99
days, stage III = 553 days and stage II > 401 days, P < .001). The results from dogs vaccinated with huTyr were published in 2003 (Bergman et al, Clin Cancer Res 2003).

**DEVELOPMENT OF SPECIFIC ANTI-TYROSINASE HUMORAL IMMUNE RESPONSES**

We have recently investigated the humoral responses of dogs receiving HuTyr as a potential explanation for the long-term survivals seen in some of the dogs on this study. Utilizing standard ELISA with mammalian expressed purified human tyrosinase protein as the target of interest (kind gift of C Andreoni & JC Audonnet, Merial, Inc.), we have found 3/9 dogs with 2-5 fold post-vaccinal humoral responses compared to pre-immune sera. We have confirmed these findings utilizing a flow-cytometric-based assay of pre- and post-vaccinal sera in permeabilized human SK-MEL melanoma cells expressing endogenous human tyrosinase. Interestingly, the three dogs with post-vaccinal anti-HuTyr humoral responses are dogs with unexpected long-term tumor control (Liao et al, Cancer Immunity, 2006). Co-Investigators have also determined that normal dogs receiving the HuTyr-based melanoma vaccine develop Ag-specific IFN-γ T cells (Goubier et al, Vaccine, 2008).

**CONCLUSIONS**

The results of these trials demonstrate that xenogeneic DNA vaccination in CMM is: 1) safe, 2) develops specific anti-tyrosinase humoral and cell-mediated immune responses, 3) potentially therapeutic with particularly exciting results in stage II/III local-regional controlled disease and dogs receiving MuTyr/HuGM-CSF combination, and 4) an attractive candidate for further evaluation in an adjuvant, minimal residual disease Phase II setting for CMM. A safety and efficacy USDA licensure multi-institutional trial investigating HuTyr in dogs with locally controlled stage II/III oral melanoma was initiated in April, 2006. Human trials of xenogeneic tyrosinase DNA vaccination have recently initiated. In March 2007 and December 2009, we received conditional followed by full licensure (respectively) from the USDA for the canine melanoma vaccine. This represents the first US-government approved vaccine for the treatment of cancer across species.

In summary, CMM is a more clinically faithful therapeutic model for HM when compared to more traditional mouse systems as both human and canine diseases are chemoresistant, radioresistant, share similar metastatic phenotypes/site selectivity, and occur spontaneously in an outbred, immuno-competent scenario. In addition, this work also shows that veterinary cancer centers and human cancer centers can work productively together to benefit veterinary and human patients afflicted with cancer.
REFERENCES:


OSTEOSARCOMA: WHAT CHEMO? WHEN?

Philip J. Bergman DVM, MS, PhD, DACVIM (Oncology)
Director, Clinical Studies – VCA Antech
Medical Director, Katonah-Bedford Veterinary Center

INCIDENCE AND RISK FACTORS
Osteosarcoma (OSA) is the most common primary bone tumor in the dog (85% of skeletal malignancies). It is estimated to occur in over 8,000 dogs/year in the United States. OSA has bimodal age incidence peaks at 18-24 months and 7 years and occurs predominately in large to giant breed dogs in the appendicular skeleton at metaphyseal sites, whereas smaller breed dogs generally have their OSA in the axial skeleton. For appendicular OSA, the saying “Away from the elbow and close to the knee” is a good generalization; however, OSA can occur at sites such as distal tibia and others. In addition OSA is seen in the oral cavity (maxilla or mandibular), nasal cavity, ribs, digits and many other bony sites. The etiology of OSA is unknown but thought to be related to traumatic microfractures.

PATHOLOGY AND BEHAVIOR
OSA is a malignant mesenchymal tumor of primitive bone cells. The presence of extracellular matrix production of osteoid helps differentiate OSA from other bone sarcomas such as chondrosarcoma, lymphosarcoma, Hemangiosarcoma, and others. There are multiple histologic sub-classifications (osteoblastic, fibroblastic, chondroblastic, telangiectatic, etc.) for OSA; however, sub-classifications do not presently appear to be prognostic. OSA causes bone lysis, production of bone, or both; pathological fractures can occur. Less than 5% of dogs present with radiographically detectable pulmonary metastasis whereas > 90% have micrometastases at presentation.

HISTORY AND CLINICAL SIGNS
Most dogs with OSA present for lameness and/or swelling at the local site if appendicular in origin. For non-appendicular OSA, the history and clinical signs are dependant on the specific site of origin.

DIAGNOSIS
Local radiographs document soft tissue swelling, bone lysis, production of bone, or a combination of both. The differential diagnosis for OSA includes: Primary or secondary bone tumor, myeloma, lymphoma, and osteomyelitis. The definitive diagnosis of OSA requires bone biopsy. While most biopsies of cancer aim for the periphery of a lesion, bone biopsies should be taken from the center of the lesion and multiple biopsies should be performed. In addition, a full physical examination with close palpation of local lymph nodes (aspirate & examine if enlarged) is also recommended. For additional staging, three view thoracic radiographs are strongly recommended. The utility of additional staging diagnostics such as bone survey radiographs and/or nuclear medicine bone scanning are somewhat controversial. A surgical staging system suggests most OSA are stage IIb (high-grade, extracompartmental and no gross metastasis).
**Therapy:** The median survival times reported for appendicular OSA are summarized in the table below.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Median DFI/MFI</th>
<th>Median Surv. Time</th>
<th>% Alive at 1 Year</th>
<th>% Alive at 2 Years</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>~ 45-60 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amputation only¹ ²</td>
<td>110-112 days</td>
<td>10-12%</td>
<td>~ 2%</td>
<td></td>
</tr>
<tr>
<td>Amputation only³</td>
<td>175 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X3 Palliative RT⁴ ⁵</td>
<td>73-122 days</td>
<td>7%</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>X4 Palliative RT⁶ (other Rx)</td>
<td>313 days</td>
<td>~ 37%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Palliative RT &amp; Carbo</td>
<td>103 days</td>
<td>25%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amp &amp; Cisplat⁷ ⁹</td>
<td>177-226 days</td>
<td>262-325 days</td>
<td>38-46%</td>
<td>16-21%</td>
</tr>
<tr>
<td>Amp &amp; Doxo q 2wk¹⁰</td>
<td>366 days</td>
<td>50.5%</td>
<td>9.7%</td>
<td></td>
</tr>
<tr>
<td>Amp &amp; Carbo¹¹</td>
<td>257 days</td>
<td>321 days</td>
<td>35.4%</td>
<td></td>
</tr>
<tr>
<td>Amp &amp; OPLA-sponge¹²</td>
<td>256 days</td>
<td>278 days</td>
<td>41.2%</td>
<td></td>
</tr>
<tr>
<td>Amp &amp; Alt Dox/Cisplat³</td>
<td>210 days</td>
<td>300 days</td>
<td>37%</td>
<td>26%</td>
</tr>
<tr>
<td>Amp &amp; combo Dox/Carbo¹³</td>
<td>195 days</td>
<td>235 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amp &amp; Dox/Cispl combo¹⁴</td>
<td>470 days</td>
<td>540 days</td>
<td>68.7%</td>
<td>25%</td>
</tr>
<tr>
<td>Followup Dox/Cispl</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amp &amp; Dox/Cispl (vcs abst)</td>
<td>330 days</td>
<td>300 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amp &amp; MTP &amp; Cispl¹⁵</td>
<td>189-336 days</td>
<td>315-432 days</td>
<td>~ 40%</td>
<td>0 ~ 20%</td>
</tr>
<tr>
<td>Amp &amp; lipo-Cispl¹⁶</td>
<td>156 days</td>
<td>333 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amp &amp; Lobaplatin¹⁷</td>
<td></td>
<td></td>
<td>31.8%</td>
<td></td>
</tr>
<tr>
<td>Amp &amp; altern Carbo/Dox¹⁸</td>
<td>227 days</td>
<td>320 days</td>
<td>48%</td>
<td>18%</td>
</tr>
</tbody>
</table>

**Prognostic Factors¹⁹ ²⁷**

**Better**
- No overt metastasis
- ↑↑ tumor necrosis (neoadjuvant chemotherapy only)
- Mandibular location

**Worse**
- < 5 yr age
- ↑↑ tumor size
- Proximal humerus
- > 40 kg weight
- ↑ ALP
- ↑↑ grade
REFERENCES:


27. Selvarajah GT, Kirpensteijn J Prognostic and predictive biomarkers of canine osteosarcoma, *Vet J.* 2010;


