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MELANOMA REVIEW

Melanomas in dogs have extremely diverse biologic behaviors depending on a variety of factors. A greater understanding of these factors significantly helps the clinician to delineate in advance the appropriate staging, prognosis and treatments. The primary factors which determine the biologic behavior of a melanoma in a dog are site, size, stage and histologic parameters. Unfortunately, even with an understanding of all of these factors, there will be occasional melanomas which have an unreliable biologic behavior; hence the desperate need for additional research into this relatively common (~ 4% of all canine tumors), heterogeneous, but frequently extremely malignant tumor. This review will assume the diagnosis of melanoma has already been made, which in of itself can be fraught with difficulty, and will focus on the aforementioned biologic behavior parameters, the staging and the treatment of canine melanoma.

Biologic Behavior

The biologic behavior of canine melanoma is extremely variable and best characterized based on anatomic site, size, stage and histologic parameters. On divergent ends of the spectrum would be a 0.5 cm haired-skin melanoma with an extremely low grade likely to be cured with simple surgical removal vs. a 5.0 cm high-grade malignant oral melanoma with a poor-grave prognosis. Similar to the development of a rational staging, prognostic and therapeutic plan for any tumor, two primary questions must be answered; what is the local invasiveness of the tumor and what is the metastatic propensity? The answers to these questions will determine the prognosis, and to be discussed later, the treatment.

Site

The anatomic site of melanoma is highly, though not completely, predictive of local invasiveness and metastatic propensity. Melanomas involving the haired-skin which are not in proximity to mucosal margins often behave in a benign manner. Surgical removal through a lumpectomy is often curative, but histopathological examination is imperative for delineation of margins as well as a description of cytologic features. In haired-skin melanomas exhibiting histopathologic criteria of malignancy, the reader is referred to the grade discussion below. Oral and/or mucosal melanoma has been routinely considered an extremely malignant tumor with increased local invasiveness and high metastatic propensity. This biologic behavior is extremely similar to human oral and/or mucosal melanoma. Melanoma is the most common oral tumor in the dog; additional neoplastic differentials include squamous cell carcinoma, fibrosarcoma, epulides/odontogenic tumors and others. In canine oral/mucosal melanomas with histological reporting suggestive of a benign lesion, the reader is referred to the grade discussion below. The anatomic sites that split the opposite ends of the prognostic spectrum of generally benign-acting haired-skin vs. typically malignant and metastatic oral/mucosal melanomas include melanomas of the digit and foot pad. Dogs with melanoma of the digits without lymph node or further metastasis treated with digit amputation are reported to have median survival times of ~ 12 months, with 42-57% alive at 1 year and 11-13% alive at 2 years. Unfortunately, metastasis from digit melanoma at presentation is reported to be ~ 30-40%, and the aforementioned outcomes with surgery suggest that subsequent distant

metastasis is common even when no metastasis is found at presentation/digit amputation. The prognosis for dogs with melanoma of the foot pad has not been previously significantly reported; this author has found this anatomic site to be similar in metastatic propensity and prognosis to digit melanoma. Interestingly, human acral lentiginous melanoma (plantar surface of the foot, palms of the hand and digit) has an increased propensity for metastasis.

Size and Stage

For dogs with oral melanoma, primary tumor size has been found to be extremely prognostic. The WHO staging scheme for dogs with oral melanoma is primarily based on size, with stage I = < 2 cm. diameter tumor, stage II = 2 cm. to < 4 cm. diameter tumor, stage III = 4 cm. or greater tumor and/or lymph node metastasis and stage IV = distant metastasis. MacEwen and colleagues reported median survival times (MST) for dogs with oral melanoma treated with surgery to be approximately 17-18, 5-6 and 3 months with stage I, II and III disease, respectively. More recent reports suggest stage I oral melanoma treated with standardized therapies including surgery, radiation and/or chemotherapy have a MST of approximately 12-14 months, with most dogs dying of distant metastatic disease, not local recurrence.

A variety of limitations exist with the present WHO staging scheme for canine oral melanoma. First, the size of the tumor is not standardized to the size of the patient. Therefore, a 1.8 cm oral melanoma without lymph node metastasis is a stage I melanoma in a Rottweiler, as well as a Chihuahua. Further investigations with standardization to patient size are hereby encouraged. In addition, the histologic appearance and other histologically-based indices of melanomas are not accounted for in the present WHO staging scheme, and proposed alternate schemes incorporating histologic criteria have unfortunately not gained traction in canine melanoma. For these reasons and others, various investigators have pursued other prognostic factors in canine oral melanoma in order to possibly develop alternative staging systems. These investigations have continued to find size to be extremely prognostic, but have also found the following negative prognostic factors: lesser degree of removal and incomplete surgical margins, location (caudal mandibular and rostral maxillary do more poorly), tumor mitotic index > 3, and bone invasion/lysis. Prospective investigations including these variables into an expanded WHO staging system are hereby encouraged.

The staging system for canine non-oral melanoma is remarkably less well defined to date. Henry and colleagues utilized the WHO TNM system for canine digital tumors, which defines T1 = tumor < 2 cm and superficial, T2 = tumor 2-5 cm. and minimum invasion, T3 = tumor > 5 cm. or invading subcutis and T4 = tumor invading fascia or bone. They reported that metastasis free interval was significantly inversely associated with T stage across all digit tumors. When specifically examining dogs with digit melanoma, there was 1 dog with T2, 5 dogs with T3 and 4 dogs with T4 tumors. Further studies defining staging schemes for canine non-oral melanoma with clinical variables and outcomes are also encouraged.

Grade and Histologic Parameters

Histopathologic grading of a tumor by the pathologist delineates degree of malignancy and grading systems vary across tumor types. The histological grade is commonly predictive of survival, metastatic rate and other clinical variables in a wide variety of tumors across species, including canine melanoma. For example, in haired-skin melanomas exhibiting multiple histopathologic criteria of malignancy, such as increased mitotic rate, invasiveness and/or poor differentiation, metastatic propensity is increased and the prognosis is reduced due to variability in outcomes post-operatively. Bostock et al reported that 45% of dogs with malignant skin melanomas died within one year whereas 8% "benign" skin melanomas died from their disease. Furthermore, 10% of dogs with haired-skin melanoma with a mitotic index of 2 or less died from their tumor 2 years after surgery compared to > 70% dogs dying from a tumor with a mitotic index of 3 or more. Dogs with haired-skin melanomas within 1 cm of mucosal margins have been

minimally investigated to date; this author has had multiple patients with histologically benign, haired-skin melanoma within 1 cm. of a mucosal margin develop subsequent distant metastatic disease.

The most exhaustive review of histologic findings in canine melanocytic neoplasms was published by Spangler and Kass. In this paper, 384 dogs with melanoma or melanocytoma had their tumors comprehensively histologically examined and statistically tested for association with malignant behavior (recurrence and/or metastasis) and median survival time via follow-up provided by the veterinarians submitting the samples. Significant negative prognostic factors included metastasis (i.e. stage as discussed above), size/tumor volume and a variety of histologic criteria such as mitotic index, nuclear atypia, tumor score, presence of deep inflammation, intralesional necrosis and junctional activity. As expected, these investigators also found three primary anatomic-location mortality groups: 1) oral (19% of samples), 2) feet and mucosal surface of lips (19% of samples) and 3) cutaneous (59% of samples). Too few ocular melanomas were investigated to make recommendations. An unexpected finding from this investigation was the presence of 32% of dogs with oral melanoma without malignant behavior according to their criteria (no recurrence, no metastasis and alive at the end of study or dead due to competing causes). This author sees no reason why oral melanomas may not occasionally behave in a benign fashion; however, 32% is a significantly different enough frequency from all previous reports (which report a very small proportion to no benign oral melanomas) to warrant additional study. Similarly, the number of benign acting oral melanomas was relatively small (n=22) and a variety of factors such as lack of necropsy, type of follow-up, lack of reporting of number of lost to follow-up cases and lastly the large number of cases disqualified for inclusion because of poor differentiation, may have led to an increased frequency of "benign behaving" cases. Similarly, this author has seen in excess of 20 dogs in the last 7 years with a previous histopathologic diagnosis of benign oral melanoma presenting with distant metastasis. This is consistent with Bostock et al reporting three of seven dogs with "benign" oral melanoma going on to die of their disease.

Spangler and Kass also reported that 38% and 12% of feet/mucosal surface of lips and cutaneous melanocytic tumors, respectively, behaved in a malignant fashion. Four percent and 27% of those dogs that died of a foot/lip and cutaneous melanoma, respectively, had a tumor score which would have predicted benign behavior. Upon further review of those cases, there were no attributes found that would allow for prediction of malignant behavior. This suggests that additional testing is needed beyond routine light microscopy for delineation of malignant vs. benign behavior for canine cutaneous melanoma. Laprie et al reported the use of Ki-67 expression via immunohistochemistry in 68 canine cutaneous melanomas. This group found that the predictive value of Ki-67 proliferative index (97%) was greater than the predictive value of classical histology (91%) for biologic behavior in canine cutaneous melanoma. This strongly suggests that the use of Ki-67 immunohistochemistry and possibly other proliferative markers (e.g. AgNOR and others) in canine cutaneous melanoma should be commonly performed after the histopathologic diagnosis is made.

Staging

The staging of dogs with melanoma is relatively straightforward. A minimum database should include a thorough history and physical exam, complete blood count & platelet count, biochemical profile, urinalysis, 3 view chest films and local lymph node aspiration (ipsilateral and contralateral nodes for oral melanoma due to variability in draining patterns) with cytology whether lymphadenomegaly is present or not. Williams & Packer reported in dogs with oral melanoma that ~ 70% had metastasis when lymphadenomegaly was present, but more importantly ~ 40% had metastasis when no lymphadenomegaly was present. Additional considerations should be made for abdominal compartment testing (e.g. abdominal ultrasound) in all cases of canine melanoma, especially in cases with potentially moderately to highly metastatic anatomic sites such as the oral cavity, feet or mucosal surface of the lips, as melanoma may metastasize to the abdominal lymph nodes, liver, adrenal glands and other sites.

Treatment

The treatment for dogs with melanoma without distant metastatic disease on staging starts with local tumor control. This is generally best completed through surgical extirpation due to its speed, increased curative intent and reduced cost compared to other modalities. The dose of surgery is generally based on the anatomic site of the melanoma, with cutaneous melanomas usually requiring lumpectomy and all other sites requiring more aggressive and wide excision. While large resections such as partial mandibulectomy or maxillectomy carry an inherent level of morbidity, excellent outcomes & owner satisfaction rates are routinely 90% or greater. It cannot be overstated the importance of complete staging when contemplating larger resections; the presence of distant metastatic disease would attenuate the use of more radical surgical procedures and convert the patient to medical and/or palliative care options.

Radiation therapy (RT) plays a role in the treatment of canine melanoma when the tumor is not surgically resectable, the tumor has been removed with incomplete margins and/or the melanoma has metastasized to local lymph nodes without further distant metastasis. The use of smaller fractions of RT (e.g. 3-4 Gy) given daily to every other day can allow for a greater total dose and
fewer chronic RT reactions; however, melanoma appears somewhat resistant to these types of fractionation schemes. Coarse fractionation schemes for canine melanoma utilizing 6-9 Gy weekly to every other week to a total dose of 24-36 Gy have been reported by a variety of investigators with complete remission rates of 53-69% and partial remission rates of 25-30%. Unfortunately, recurrence and/or distant metastasis was common in all of these studies. Other modalities reported for local tumor control as case reports and/or case series have included intralesional cisplatin implants, intralesional bleomycin with electronic pulsing and many others, but widespread use has not been reported to date and recurrence rates appear high.

In dogs with melanoma in the aforementioned anatomic sites predicted to have a moderate to high metastatic propensity, or dogs with cutaneous melanoma with a high tumor score and/or increased proliferation index through increased Ki-67 expression, the use of systemic therapies is warranted. Rassnick and colleagues reported an overall response rate of 28% using carboplatin for dogs with malignant melanoma. Unfortunately, only one dog had a minimally durable complete response, and the rest were non-durable partial responses. Similarly, Boria et al reported an 18% response rate and median survival time of 119 days with cisplatin and piroxicam in canine oral melanoma. Other reports using single agent dacarbazine, melphalan or doxorubicin suggest poor to dismal activity. More recently and importantly, two studies (Proulx et al and Murphy et al) suggest that chemotherapy plays an insignificant role in the adjuvant treatment of canine melanoma. It can be argued that the studies performed to date to evaluate the activity of chemotherapy in an adjuvant setting for canine melanoma have been suboptimal due to a variety of reasons; however, the extensive human literature in this specific setting suggests melanoma is an extremely chemotherapy resistant tumor. It is clear that new approaches to the systemic treatment of this disease are desperately needed.

Immunotherapy represents one potential systemic therapeutic strategy for melanoma. A variety of immunotherapeutic strategies for the treatment of human melanoma have been reported previously, with typically poor outcomes due to a lack of breaking tolerance. Immunotherapy strategies to date in canine melanoma have used autologous tumor cell vaccines (with or without transfection with immunostimulatory cytokines and/or melanosomal differentiation antigens), allogeneic tumor cell vaccines transfected with interleukin 2 or GM-CSF, liposomal-encapsulated non-specific immunostimulators (e.g. L-MTP-PE), intralesional Fas ligand DNA, bacterial super-antigen approaches with granulocyte macrophage colony-stimulating factor or interleukin 2 as immune adjuvants and lastly canine dendritic cell vaccines loaded with melanosomal differentiation antigens. Although these approaches have produced some clinical anti-tumor responses, the methodologies for the generation of these products are expensive, time consuming, sometimes dependent on patient tumor samples being

established into cell lines and fraught with the difficulties of consistency, reproducibility, and other quality control issues.

The advent of DNA vaccination circumvents some of the previously encountered hurdles in vaccine development. DNA is relatively 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. 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 (different species) 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.

What is the latest information on the commercially available melanoma vaccine that Dr. Bergman and colleagues at Memorial Sloan-Kettering developed?

With the melanoma vaccine, we targeted defined melanoma differentiation antigens of the tyrosinase family. Tyrosinase is a melanosomal glycoprotein, essential in melanin synthesis. Immunization with xenogeneic (i.e. different species) human DNA encoding tyrosinase family proteins induced antibodies and cytotoxic T-cells against melanoma cells in mice, but immunization with mouse tyrosinase-related DNA did not induce detectable immunity. Thus, xenogeneic DNA vaccination could break tolerance against a self-tumor differentiation antigen, inducing antibody, T-cell and anti-tumor responses. Therefore, we examined the use of human tyrosinase, murine gp75, murine tyrosinase and murine tyrosinase + human GM-CSF in dogs with advanced malignant melanoma (Bergman et al 2003 & 2008). We also investigated the humoral responses of dogs receiving HuTyr as a potential explanation for the long-term survivals seen in some of the dogs on these studies. 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 found 3/9 dogs with 2-5 fold post-vaccinal humoral responses compared to pre-immune sera. These findings were confirmed 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 Imm 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).

The results of these trials demonstrate that xenogeneic DNA vaccination in canine malignant melanoma is: (1) safe, (2) leads to the development of specific anti-tyrosinase immune responses, (3) is potentially therapeutic, especially with stage II/III local-regional controlled disease and (4) is an attractive candidate for further evaluation in an adjuvant, minimal residual disease Phase II setting. Consequently, 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 across 5 sites. In late March 2007, we received conditional licensure from the USDA-CVB (United States Department of Agriculture – Center for Veterinary Biologics) for the HuTyr-based canine melanoma vaccine and it became commercially available for use by US-based veterinary oncologists in June, 2007. This represented the first US-government approved vaccine for the treatment of cancer. Based on results of the aforementioned 5-site efficacy trial documenting a statistically significant improvement in survival for vaccinates vs. controls (Grosenbaugh et al, AJVR 2011), full licensure for the HuTyr-based canine melanoma vaccine was awarded by the USDA-CVB in December, 2009, which Merial (the license-holder) marketed as Oncept.

Subsequently, we have examined the efficacy of local tumor control and use of MuTyr-based DNA vaccination in dogs with digit melanoma. We found an improvement in survival compared to historical outcomes with digit amputation only and also documented a decreased prognosis for dogs with advanced stage disease and/or increased time from digit amputation to the start of vaccination (Manley et al, JVIM 2011).

A similar approach has been used in human patients with metastatic melanoma in the minimal residual disease setting. Although no clinical response data are available since these patients did not have measurable disease, several phase I trials of xenogeneic DNA vaccines have been completed. Across studies of tyrosinase and gp100 DNA immunization, approximately 40% of human patients develop quantifiable CD8+ T cell responses to the syngeneic human target antigen. Two additional exciting new approaches which appear to confer a survival benefit in human metastatic melanoma include the use of the anti-CTLA-4 antibody, ipilimumab (YervoyTM, Bristol-Myers Squibb) and the selective BRAF inhibitors, vemurafenib (ZelborafTM, Genetech) and dabrafenib (GSK2118436, GlaxoSmithKline), in patients who are BRAF V600 mutation positive.

It is hoped in the future that this same vaccine may also play roles in the treatment of melanoma in other species (e.g. horses, cats, etc.) due to its xenogeneic origins (Phillips et al AJVR 2012), and in melanoma prevention once the genetic determinants of melanoma risk in dogs are further defined.

Summary

In summary, the future is looking brighter for canine melanoma on multiple fronts. We have a greater understanding of the prognostic aspects of this disease and we now have a commercially available vaccine for treatment. It is hoped in the future that this same vaccine may also play roles in the treatment of melanoma in other species (e.g. horses, cats, humans, etc.) due to its xenogeneic origins, and in melanoma prevention once the genetic determinants of melanoma risk in dogs are further defined. It is easy to see how the veterinary oncology profession is uniquely able to greatly contribute to advances for both canine as well as human melanoma, in addition to many other cancers with similar comparative aspects across species. This author and the fields of veterinary tumor immunotherapy and veterinary oncology are greatly indebted to the tireless work and seeds laid by the late Dr. Greg MacEwen; he is greatly missed and this review is dedicated in his honor.

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