

**VCS/ACVP Oncology-Pathology Working Group
Consensus on the Diagnosis of and Histopathologic Prognostication for Canine
Melanocytic Neoplasms**

Species/Tumor: **Canine Melanoma Subgroup**

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CONSENSUS:

Based on review of the recent literature (publications reviewed listed below) the **Canine Melanoma Subgroup** has concluded and recommends the following regarding **diagnosis of, and prognostic markers for, canine melanomas**. It is expected that this document will be updated regularly as additional research and associated data are published.

Summary of Recommendations for Diagnosis and Histopathologic Prognostication of Canine Melanocytic Neoplasms:

1. The first step in prognostication of canine melanocytic neoplasms is to confirm melanocytic origin, either by identifying classic histologic features of melanocytic neoplasms or by demonstrating labeling of neoplastic cells in amelanotic neoplasms for melanocytic specific immunohistochemical markers that have been validated and published in dogs.
 - a. Classic histologic features of melanocytic neoplasms: intracytoplasmic melanin, variable cell morphology in the same tumor, junctional activity, pagetoid growth, presence of neoplastic cells at the mucosal-submucosal (epidermal-dermal) junction even in the absence of junctional activity, and finely stippled to vesiculate nuclei with a prominent central nucleolus (“owl’s eye”)
 - b. If amelanotic or otherwise poorly differentiated, demonstrate immunohistochemical labeling for Melan-A, PNL2, TRP-1 or TRP-2
2. Determine prognosis of both cutaneous and lip/oral melanocytic neoplasms by evaluating the specific histologic and molecular parameters described in the Table 1 below. For heavily pigmented neoplasms, bleaching may be necessary in order to accurately evaluate nuclear features and mitotic count. To evaluate Ki67 labeling in heavily pigmented neoplasms, bleaching should be performed AFTER immunohistochemical labeling for Ki67.
 - a. For lip/oral melanocytic neoplasms:
 - i. if there is marked nuclear atypia ($\geq 30\%$ atypical nuclei), a high mitotic count* [$\geq 4/10$ hpf (400x magnification/40x objective) or, ideally, in an area of 2.37mm^2], or evidence of vascular invasion or metastasis, the neoplasm is diagnosed as a malignant melanoma and evaluation of the Ki67 index is offered for further prognostication [pathologists may indicate in a report that Ki67 index is not necessary for neoplasms that greatly surpass the nuclear atypia and mitotic count thresholds and/or show evidence of vascular invasion or metastasis; there are no established values for “greatly surpassing” the cut-offs, but pathologists will use their experience to subjectively make this recommendation. In general, the subgroup agrees that Ki67 index is not necessary for tumors with $\geq 50\%$ atypical nuclei or a mitotic count $\geq 40/10$ hpf (400x magnification/40x objective or, ideally, in an area of 2.37mm^2) which is 10 times higher than the threshold value.]
 - ii. if the histologic parameters give mixed results, are at or near the threshold values, or if all the histologic parameters indicate a favorable prognosis, evaluate the Ki67 index for further prognostication
 - iii. Ki67 index is the most specific parameter for prognostication
 - iv. provide the results of all parameters examined in the pathology report
 - b. For cutaneous melanocytic neoplasms:
 - i. If there is marked nuclear atypia ($\geq 20\%$ atypical nuclei), a high mitotic count* [$\geq 3/10$ hpf (400x magnification/40x objective) or, ideally, in an area of 2.37mm^2], a tumor thickness >0.95 cm, or evidence of vascular

invasion or metastasis, the neoplasm is diagnosed as a malignant melanoma and evaluation of the Ki67 index is offered for further prognostication [pathologists may indicate in a report that Ki67 index is not necessary for neoplasms that greatly surpass the nuclear atypia and mitotic count thresholds and/or show evidence of vascular invasion or metastasis; there are no established values for “greatly surpassing” the cut-offs, but pathologists will use their experience to subjectively make this recommendation. In general, the committee agrees that Ki67 index is not necessary for tumors with $\geq 50\%$ atypical nuclei or a mitotic count $\geq 30/10$ hpf (400x magnification/40x objective or, ideally, in an area of 2.37mm^2) which is 10 times higher than the threshold value.]

- ii. if the histologic parameters give mixed results, are at or near the threshold values, or if all the histologic parameters indicate a favorable prognosis, evaluate the Ki67 index for further prognostication
 - iii. Ki67 index is the most specific parameter for prognostication
 - iv. provide the results of all parameters examined in the pathology report
3. Some general rules for prognostication of melanocytic neoplasms are listed below:
- a. It is impractical to accurately predict, on an individual basis, the biological behavior of melanocytic neoplasms
 - b. More than one parameter should be used to classify melanocytic neoplasms histologically as benign, of low malignant potential, or malignant because of the inherent subjectivity in histological evaluation
 - c. If histologic prognostic factors conflict with one another, a neoplasm should be diagnosed as a melanocytic neoplasm and prognostic factors should be discussed
 - d. Evaluation of nuclear atypia and mitotic count in combination with Ki67 index and clinical features will maximize the percentage of correctly classified neoplasms ^{Smedley 2011b}

*For this consensus, the mitotic count (reported as mitotic index, in the literature reviewed) is obtained by counting the absolute number of mitoses in 10 high-power fields (400x magnification/40x objective) in the region with highest mitotic activity, as determined initially on a low power scan (100x magnification/10x objective) of the specimen. Future studies which evaluate mitotic count as a prognostic parameter should also adopt this methodology, while also defining and standardizing the microscopic area evaluated to 2.37mm^2 .

**Table 1. Prognostication of Canine Cutaneous and Oral/Lip Melanocytic Neoplasms
(modified from Smedley et al. 2011b)**

Location	oral/lip melanocytic neoplasms	cutaneous/digit melanocytic neoplasms	
Distant metastasis	poor prognosis	poor prognosis	
Lymphatic invasion	poor prognosis	poor prognosis ^a	
Mitotic count^b	10 consecutive fields starting in area w/ highest mitotic activity	10 consecutive fields starting in area w/ highest mitotic activity	
	avoid areas of ulceration for both methods		
	<4/10hpf	favorable prognosis	<3/10hpf
	≥4/10hpf	poor prognosis	≥3/10hpf
Nuclear atypia^c	% atypical nuclei	% atypical nuclei	
	<30%	favorable prognosis	<20%
	≥30%	poor prognosis	≥20%
Degree of pigmentation	subjective assessment		
	% pigmented cells	scale 0 (no pigment) to 2 (high pigment)	
	≥50%	favorable prognosis	2
	<50%	uncertain prognosis	0 to 1
Presence of ulceration	no prognostic significance	poor prognosis	
Level of infiltration/invasion	Shallow or raised w/ no bone lysis	favorable prognosis	Limited to dermis
	deep w/ possible bone lysis	poor prognosis	Extends beyond dermis
Tumor thickness	Not investigated	favorable prognosis	≤0.95cm tumor thickness ^d
		poor prognosis	>0.95cm tumor thickness ^d
Ki67 index	average number of positive nuclei per grid (5 hpf grid areas counted)	% of positive nuclei in 500 cells counted	
	avoid areas of ulceration & inflammation and assess highest staining areas for both methods		
	<19.5	favorable prognosis	<15%
	≥19.5	poor prognosis	≥15%

^aParameter was not specifically examined for neoplasms of the digit

^bFor this consensus, the mitotic count (reported as mitotic index, in the literature reviewed) is obtained by counting the absolute number of mitoses in 10 high-power fields (400x magnification/40x objective or, ideally, in an area of 2.37mm²) in the region with highest mitotic activity, as determined initially on a low power scan (100x magnification/10x objective) of the specimen.

^cParameter should be assessed in epithelioid predominant neoplasms and in spindle neoplasms with sufficiently observable nuclear detail

^dTumor thickness is measured with a ruler by placing the ruler on the glass slide perpendicular to the epidermis or mucosal epithelial surface and measuring the largest thickness of the tumor.^{Silvestri}

*A favorable prognosis relates to expected survival times longer than one year and a poor prognosis relates to an expected death due to melanocytic neoplasia within less than one-year post-diagnosis for all melanocytic neoplasms. These predictions are based solely on publications that met our strict criteria for inclusion for each single parameter. These predictions do not take in to account stage of disease or treatment strategies. When there are mixed results, results for each parameter should be reported, but Ki67 index should be used for final interpretation in most cases.

Diagnosis of Melanocytic Neoplasms

Histologic Features of Melanocytic Neoplasms

The first step in prognostication, to assist in determining appropriate treatment strategies for melanocytic neoplasms, is to obtain an accurate diagnosis. This should also be the first step when performing and reviewing prognostic studies. If melanocytic origin has not been definitively confirmed for every neoplasm in a study population, the results of that study cannot be interpreted accurately. If histologic features alone are not confirmatory of melanocytic origin, demonstration of immunohistochemical (IHC) labeling for specific melanocytic markers is needed. Melanocytic IHC markers have different sensitivities and specificities^{Smedley 2011a}, which is discussed below.

Malignant melanomas can histologically resemble a large number of other neoplasms, including those of leukocytic origin, epithelial origin, and mesenchymal origin. Thus, diagnosis of a poorly differentiated malignant melanoma can be challenging. Histologic features that are commonly seen in melanocytic neoplasms include: intracytoplasmic melanin, variable cell morphology in the same tumor, junctional activity, pagetoid growth, presence of neoplastic cells at the mucosal-submucosal (epidermal-dermal) junction even in the absence of junctional activity, and finely stippled to vesiculate nuclei with a prominent central nucleolus (“owl’s eye”). The likelihood of obtaining an accurate diagnosis, especially for oral melanocytic neoplasms, is dramatically increased when there is sufficient overlying epithelium, as well as flanking lateral epithelium, within the submitted sample. **When performing incisional biopsies, clinicians are encouraged to submit non-ulcerated regions of the mass as well as samples of the adjacent intact surface epithelium. When performing excisional biopsies, clinicians should take wide lateral surface epithelial margins, especially for oral/lip neoplasms. These strategies will improve the chances of finding evidence of neoplastic cells at the mucosal-submucosal (epidermal-dermal) junction, junctional activity, and/or pagetoid growth. Excisional biopsy with wide margins will also improve the likelihood of complete excision, due to the tendency of melanocytic neoplasms to exhibit lateral intraepithelial spread. In addition, intraepithelial nests of neoplastic cells are also the most likely cells to label with melanocytic immunohistochemical (IHC) markers.**^{Smedley 2011a} Amelanotic malignant melanomas can have several differentials and IHC labeling is needed for further differentiation in these cases.

In addition, cytologic examination +/- immunocytochemical (ICC) examination of potential melanocytic neoplasms should not be forgotten as a potential useful preoperative diagnostic tool.^{Przedziecki} In one study, the authors found that ICC using anti-Melan-A, anti-Vimentin, and anti-cytokeratin improved the ability to diagnose canine amelanotic oral melanomas with cytology. In this small study, the diagnosis reached with ICC matched the histologic diagnosis.^{Przedziecki} The study also showed that ICC for Melan A can correctly identify metastatic amelanotic melanoma in regional lymph nodes, especially when they are in low numbers or have a round cell morphology.^{Przedziecki} It still may be difficult to differentiate between metastatic neoplastic melanocytes and draining melanocytes, however.

Immunohistochemical Features of Melanocytic Neoplasms

Melanocytic neoplasms in veterinary species have been shown to label for several different IHC markers, but each marker has different sensitivities and specificities among

species, including humans, thereby limiting the use of a single antibody for diagnosing amelanotic melanocytic neoplasms. The most sensitive and specific markers to detect melanocytic neoplasms in veterinary species are Melan-A and PNL2 ^{Smedley 2011a}, which are antigens that are found on melanocytes. In dogs, antibodies against tyrosinase-related proteins 1 and 2 (TRP-1 and TRP-2) have also been shown to be highly sensitive and specific. ^{Smedley 2011a} A diagnostic melanoma cocktail that contains antibodies against Melan-A, PNL2, TRP-1 and TRP-2 has been shown to have 100% specificity and 93.9% sensitivity in detecting canine oral melanocytic neoplasms compared to soft tissue sarcomas in one study. ^{Smedley 2011a} This cocktail has been shown to have a sensitivity that is greater than the individual sensitivities of each individual antibody and to result in a greater labeling intensity. ^{Smedley 2011a} Thus, this cocktail makes it easier to identify labeling in small samples and in tumors that only exhibit a small amount of labeling with the individual markers. The most common cells to label with melanocytic markers are intraepithelial neoplastic cells, which are the most differentiated of the neoplastic cells, as the growth of the neoplasm begins in the epithelium. ^{Smedley 2011a} **Thus, again, it is extremely important for clinicians to submit non-ulcerated portions of the mass, as well as wide lateral margins that include intact lateral flanking epithelium, in order to increase the likelihood of identifying intraepithelial nests of neoplastic melanocytes.** Small nests can sometimes be difficult to discern with routine histology, but they are easily identified with IHC labeling.

Other markers that have a high sensitivity for detecting canine melanocytic neoplasms include S-100 and MiTF. ^{Smedley 2011a} These markers are commonly used in the detection of human melanocytic neoplasms where soft tissue sarcomas have a much lower incidence than in dogs. In dogs, soft tissue sarcomas have a high prevalence and often label for S-100 and/or MiTF. Thus, these markers have a much lower specificity (20% and 30% respectively in one study) in dogs and are not useful for differentiating between a spindle malignant melanoma and a soft tissue sarcoma. ^{Smedley 2011a} These markers should never be used as sole markers for diagnosing melanocytic origin in routine diagnostics or in research studies. HMB-45, tyrosinase, and tyrosine hydroxylase (TH) are additional markers that are used in human medicine for the diagnosis of melanocytic neoplasms. While specific, in dogs HMB-45 has been shown to have varying sensitivities depending on the study performed and tyrosinase and TH have been shown to have very low sensitivities for detecting canine melanocytic neoplasms. ^{Smedley 2011a} Thus, these markers are not recommended in dogs.

SOX-10 is a marker that has been used to diagnose melanocytic neoplasms in humans, ^{Miettinen, Behrens} but there are no published studies of its use in dogs. In humans, it is not specific to melanocytes and has been shown to be consistently expressed in benign Schwann cell tumors of soft tissue and the GI-tract and to be variably present in malignant peripheral nerve sheath tumors. ^{Miettinen} It has also been shown to label myoepithelial cell origin tumors, granular cell tumors, histiocytes, some epithelial tumors, occasional alveolar rhabdomyosarcomas, and rare squamous carcinomas of the head and neck and pulmonary small cell carcinomas. ^{Miettinen, Behrens, Han J 2019, Karamchandani} In addition, Sox10 can be expressed in entrapped non-neoplastic Schwann cells or melanocytes in various neoplasms and this has to be considered when diagnosing Sox10-positive tumors. ^{Miettinen} In humans, soft tissue sarcomas are uncommon and often are not a differential for a melanoma. In contrast, soft tissue sarcomas, including peripheral nerve sheath tumors, in dogs are very common and are the

primary differential for a spindle melanoma. As there are currently no publications regarding SOX-10 labeling in canine neoplasms, peer-reviewed studies evaluating this marker in various canine neoplasms are needed.

As for any poorly differentiated neoplasm, a panel of IHC markers will always provide the most useful information. When multiple differentials exist, it is best to use markers that will label each differential.

Prognostication of Melanocytic Neoplasms

The committee critically reviewed peer-reviewed published manuscripts related to the prognostication of canine melanocytic neoplasms that were published after the 2011 review paper by Smedley et al.^{Smedley 2011b} Similar to the 2011 review paper by Smedley et al.,^{Smedley 2011b} the committee used the standards outlined by the “Recommended Guidelines for the Conduct and Evaluation of Prognostic Studies in Veterinary Oncology” by Webster et al. (2011)^{Webster} to evaluate those manuscripts. The recommendations in the review paper by Smedley et al. (2011)^{Smedley 2011b} were judged to still be the most diagnostically useful, with the addition of assessing tumor thickness for cutaneous melanocytic neoplasms. A small number of additional prognostic markers have been evaluated since publication of this review paper and a few of them appear to be useful for prognostication. However, none of them have been proven in routine diagnostic settings and most require some additional investigation. Unfortunately, there have only been rare published prospective prognostic studies for canine melanocytic neoplasms and those particular studies have not yielded established prognostic markers at this point.

The first step in prognostication is to make an accurate diagnosis, which is discussed above. An accurate prognosis is critical for appropriate recommendations for primary and/or adjunct therapy. Assessment of a combination of highly reliable parameters will provide the most accurate prediction of prognosis. The focus of this consensus statement is on location, histological features, and molecular markers. Parameters for clinical prognostication are not addressed here, but are slated for future evaluation and consensus. It is hoped that clinical prognostic parameters in conjunction with histologic prognostic parameters will further increase the strength of prognostication of canine melanocytic neoplasms.

Historically, canine oral melanocytic tumors have been regarded as malignant and cutaneous melanocytic tumors have been regarded as benign. While, in general, digital/subungual and lip tumors have been shown to have increased recurrence and metastasis compared to tumors at other cutaneous sites^{Lacroix} other studies have shown that this is not always the case and that location alone cannot predict prognosis.^{Laver, Spangler, Esplin, Bergin, Laprie} In one study, 92% of oral and 74% of feet & lip melanocytic neoplasms were originally classified as malignant but only 59% of oral and 38% of feet & lip neoplasms showed malignant behavior, and a subset of cutaneous melanocytic neoplasms showed malignant behavior that would have been predicted to be benign based on location and current microscopic criteria for prognostication.^{Spangler} Thus, the challenge is to distinguish benign oral/lip melanocytic neoplasms and malignant cutaneous ones. Histologic criteria and Ki67 labeling are evaluated for this purpose.

In terms of histologic criteria, nuclear atypia, mitotic count, degree of pigmentation, level of infiltration/invasion and vascular invasion have been shown to be statistically relevant

for predicting prognosis of cutaneous and oral melanocytic neoplasms in dogs.⁶

Lacroux, Spangler, Esplin, Bergin, Laprie, Silvestri It should be noted that most veterinary studies incorrectly refer to mitotic count as mitotic index. Mitotic index is the number of mitotic figures/total number of cells in a defined area or volume of tumor and this has never been done in veterinary pathology. Mitotic count is the number of mitotic figures in a defined square mm area. For this consensus statement we have defined the mitotic count as the number of mitoses in 10 fields at 400x magnification/40x objective (which we will refer to as hpf throughout this text) because that is how earlier studies performed the mitotic count and therefore these terms and definition will be used throughout this report, regardless of how it is stated in the cited reference. While mitotic count is more objective than nuclear atypia, it is still subject to significant inter- and intraobserver variation, particularly because different microscopes and scanned images have slightly different sized fields of view depending on the type of ocular and the areas chosen by pathologists for counting mitoses can vary a great deal. The area 2.37mm² was proposed for animal tumors because this is the area in 10 fields of view with a 40X objective and a 10X ocular that has field number (FN) of 22 engraved in the eyepiece. An ocular with an FN 22 is the most common ocular manufactured by commercial sources for pathologists. Ulceration and tumor thickness is only predictive of prognosis for cutaneous tumors.Smedley 2011b, Laprie, Silvestri The Ki67 index has also been shown to be highly predictive of behavior for both oral and cutaneous melanocytic tumors in dogs in multiple studies, including three that were published after 2011.Smedley 2011b, Bergin, Lacroux, Laprie, Iussich, Campagne Whereas mitotic count only accounts for cells in the M phase of the cell cycle, Ki67 is a nuclear protein that is expressed in all phases of the cell cycle, except the resting phase. It is a measure of growth fraction. Ki67 index is much more objective and has been shown to have a higher predictive value than traditional histologic criteria.Bergin, Laprie Assessment of Ki67 index is especially helpful for melanocytic neoplasms that exhibit both prognostically favorable and poor histological parameters, or so called “grey zone” cases. Some neoplasms with histological criteria of malignancy, but low Ki67 index have been shown to have longer survival times than expected by histology.Bergin, Laprie

Table 1 can be used as a guide for prognostication. For each of the evaluated parameters, a favorable prognosis relates to expected survival times longer than one year and a poor prognosis relates to an expected death due to melanocytic neoplasia within less than one-year post-diagnosis for all melanocytic neoplasms. These predictions are based solely on publications that met our strict criteria for inclusion for each single parameter. These predictions do not take in to account stage of disease or treatment strategies. When there are mixed results, results for each parameter should be reported, but Ki67 index should be used for final interpretation in most cases. These parameters are discussed in more detail under the next two headings – “Canine Cutaneous Melanocytic Neoplasms” and “Canine Oral/Lip Mucosal Melanocytic Neoplasms.”

Canine Cutaneous Melanocytic Neoplasms

Histologic criteria can assist in distinguishing between cutaneous benign melanocytic neoplasms (melanocytomas) and malignant melanomas. Mitotic count, nuclear atypia, degree of pigmentation, presence or absence of ulceration, level of infiltration, and vascular invasion are the primary histologic features that have been used for prognostication of cutaneous

melanocytic neoplasms in dogs.^{Smedley 2011b,Laprie,Silvestri} In addition, based on recent studies, tumor thickness also appears to be a useful prognostic marker for cutaneous melanocytic neoplasms and should be recorded, although additional studies with increased case numbers and studies that compare this parameter to other established parameters, such as Ki67 index, are still needed to further evaluate this parameter and to determine if this parameter adds value to the other parameters.^{Lacroux,Silvestri} Tumor symmetry and growth pattern (expansive vs. infiltrative) are two additional parameters that have shown prognostic significance in one study but still require further evaluation with larger case numbers.^{Lacroux}

Despite the inter- and intraobserver variation of mitotic count, it has still shown statistical relevance in several studies and is more objective than nuclear atypia.^{Smedley 2011b,Laprie,Lacroux,Silvestri} The cutaneous mitotic count threshold was established by counting mitoses in 10 random high power fields.^{Laprie} However, in order to decrease the rate of underestimation of malignant neoplasms, mitotic count should be determined in the area of highest mitotic activity. Therefore, first scan the neoplasm at 100x magnification/10x objective to locate areas of potentially high mitotic activity. Locate a field containing one or more mitotic figures, if possible, and begin counting mitoses in that area at 400x magnification/40x objective (hpf). Count mitotic figures in 10 consecutive hpf. Avoid areas of ulceration, necrosis and inflammation when counting mitoses. For heavily pigmented neoplasms bleaching may be needed to better assess the mitotic count. The field number of the ocular used should also be reported, in order to improve the ability to compare mitotic counts between different studies.^{Meuten} Ideally, mitotic counts should now be reported per 2.37mm², which can be obtained by counting mitoses in 10 hpf with an FN22 ocular. A mitotic count of 3 or greater in 10 hpf has been associated with more aggressive behavior and shorter survival times.^{Smedley 2011b,Laprie} Neoplasms with less than 3 mitoses in 10 hpf generally exhibit benign behavior. One study showed that 50% of dogs with neoplasms with a mitotic count of ≥ 3 in 10 random hpf were alive for < 7 months while 90% of dogs with neoplasms with a mitotic count less than 3 in 10 random hpf were still alive at two years.^{Laprie}

Assessment of nuclear atypia can result in significant interobserver variation, especially if strict criteria are not applied. Nuclear atypia should be assessed according to criteria outlined by Spangler and Kass (2006)^{Spangler} who define well-differentiated nuclei as being small with a single centrally located nucleolus, and minimal clumping of chromatin. They may have condensed strands of nuclear chromatin extending from the nucleolus to the nuclear membrane or condensation of chromatin along the inner surface of the membrane. Cells that lack a nucleolus have fine and evenly dispersed chromatin at the periphery of the nucleus. Poorly differentiated nuclei have larger nucleoli of less regular shape that are eccentrically located within the nucleus. There are often multiple nucleoli that, in some cases, may be haphazardly connected to the inner surface of the nuclear membrane by thin strands of chromatin and give the appearance of a coarsely vacuolated nucleus.^{Spangler} The nuclear criteria may be very difficult, or not possible, to assess in some spindle, whorled/dendritic, or balloon/clear/signet ring cell variants of melanocytic neoplasms.^{Spangler} Using the above criteria for well-differentiated versus poorly differentiated nuclei, cutaneous melanocytic neoplasms should subjectively be determined to contain less than 20% atypical nuclei or greater than or equal to 20% atypical nuclei.^{Spangler,Silvestri} The most accurate way to do this is to count the number of atypical nuclei among 200 cells. This threshold value for semi-quantification has

been shown to have statistical significance for predicting survival times with a sensitivity of 80%, a specificity of 94.4%, a positive predictive value of 54.5%, a negative predictive value of 98.2%, and an overall correct classification of 93.3% for cutaneous melanocytic neoplasms in one study.^{Spangler} Statistical significance for this parameter, along with this stratification of this parameter, has also recently been confirmed by Silvestri et al.^{Silvestri} For heavily pigmented neoplasms bleaching may be needed to better assess nuclear features.

As for pigmentation, it is difficult to measure objectively with validated cutoff points, but a high degree of pigmentation does suggest a favorable clinical outcome for cutaneous neoplasms.^{Smedley 2011b, Laprie, Silvestri} This parameter has only been measured subjectively. One study scored pigmentation on a scale of 0 (no pigment) to 2 (highly pigmented) and showed that pigmentation was an independent prognostic factor and that heavily pigmented neoplasms had longer survival times.^{Laprie} Unfortunately, low to moderate pigmentation cannot be used to predict prognosis. Pigmentation should never be used as a sole prognostic indicator.

Ulceration is another prognostic marker that can be used for cutaneous melanocytic neoplasms. Ulceration of cutaneous melanocytic neoplasms has been associated with significantly shorter survival times and was shown to be an independent prognostic factor.^{Laprie, Silvestri}

Recently, tumor thickness has also been shown to be a useful prognostic marker for cutaneous melanocytic neoplasms in two studies, although the authors of those studies state that additional studies with larger case numbers are needed to further support its use as an independent prognostic marker.^{Lacroux, Silvestri} Nonetheless, the study by Silvestri *et al.* 2019 provides convincing statistical evidence that a greater tumor thickness is associated with shorter overall survival and disease-free time and provides an easy method to measure tumor thickness with established cut-off values that can be used in a diagnostic setting.^{Silvestri} Receiver operating characteristic (ROC) curve analysis and Youden Index identified cutoffs of 0.95 cm and 0.75 cm which were associated with a higher hazard for an unfavorable outcome and to develop recurrence/metastasis, respectively.^{Silvestri} Kaplan-Meier survival curves and log-rank tests were used to compare overall survival according to diagnosis.^{Silvestri} Via univariate analysis, dogs with greater tumor thickness had an approximately 10 times higher hazard of death and a greater than 5 times higher hazard to develop recurrence/metastasis than dogs with thinner tumors.^{Silvestri} The cutoff of 0.95 cm discriminated between favorable and unfavorable (tumor-related death) clinical outcomes (sensitivity = 100%, specificity = 86%; ROC curve analysis, AUC = 0.886; 95%CI, 0.795–0.977; P = .005). “Dogs with tumor thickness >0.95 cm had a shorter overall survival than those with tumor thickness ≤0.95 cm (P <.001). In particular, the 1-year estimated survival probability was 45.0%+18.8% for dogs with tumor thickness >0.95 cm and 100%+0% for those in which it was ≤0.95 cm, since no animals died in this group.”^{Silvestri} A “tumor thickness cutoff of 0.75 cm discriminated the possibility of having or not having recurrence/metastasis (sensitivity = 86%, specificity = 81%; ROC curve analysis, AUC = 0.886; 95% CI, 0.795–0.977; P = .005). Dogs with tumor thickness >0.75 cm had a shorter disease-free time than those with tumor thickness ≤ 0.75 (P < .001). In particular, the 1-year estimated probability of not developing recurrence/metastasis was 54.7%+15.4% and 97.2%+2.7% for dogs with tumor thickness >0.75 cm and ≤0.75 cm, respectively.” One must remember that false positives and false negatives can still occur with these thresholds and the prognostic significance of tumor thickness was not able to be confirmed with multivariable analysis in this

study nor was it compared to Ki67 index.^{Silvestri} In addition, the tumor thickness cutoff of 0.45cm was determined by ROC curve analysis to distinguish cutaneous malignant melanomas from melanocytomas as defined by histopathology with a sensitivity of 87% and a specificity of 64%.^{Silvestri} However, the authors stated that these values are not optimal due to the possibility of false-positive results. The authors used both an ocular micrometer as well as a standard ruler to measure tumor thickness and found that these two methods had excellent agreement. Thus, it is recommended for routine diagnostics to measure tumor thickness by simply applying a ruler to the surface of a glass slide perpendicularly to the epidermis and measuring the largest thickness of the tumor.^{Silvestri} This study also examined the usefulness of a modified Clark level measurement for predicting prognosis; however, this measurement was not associated with clinical outcome or presence of recurrence/metastasis and did not show prognostic significance.^{Silvestri} In summary, in conjunction with the other described prognostic parameters, pathologists should begin to report tumor thickness for cutaneous melanocytic neoplasms and use the cut-offs of 0.95cm and 0.75cm as one means of predicting survival times and risk of recurrence/metastasis, respectively. This will allow for greater data collection regarding this parameter.

Multiple histologic parameters should be used to classify a melanocytic neoplasm. When these parameters conflict with one another, both the favorable and poor prognostic factors should be discussed. When a cutaneous melanocytic neoplasm has marked nuclear atypia ($\geq 20\%$ atypical nuclei), a high mitotic count ($\geq 3/10$ hpf), and a tumor thickness >0.95 cm, or evidence of vascular invasion or metastasis, the neoplasm should be diagnosed as a malignant melanoma and evaluation of the Ki67 index should be offered for further prognostication. Pathologists may indicate in a report that Ki67 index is not necessary for neoplasms that greatly surpass the nuclear atypia and mitotic count thresholds and/or show evidence of vascular invasion or metastasis. There are no established values for “greatly surpassing” the cut-offs, but pathologists may use their experience to subjectively make this recommendation. In general, the committee agrees that Ki67 index is not necessary for tumors with $\geq 50\%$ atypical nuclei or a mitotic count $\geq 30/10$ hpf, which is 10 times higher than the threshold value. When these features are lacking; nuclear atypia, mitotic count, and tumor thickness are below or near the established threshold values for malignancy; or if these parameters are disparate; evaluation of the Ki67 index should be performed.

In cutaneous melanocytic neoplasms, the Ki67 index is determined as a percentage by counting the number of positively labeled melanocyte nuclei among 500 cells in the highest labeling area at 400x magnification/40x objective (hpf).^{Lacroux,Laprie} Assistance of a 1 cm² optical grid reticle is very helpful. The grid reticle simply helps the pathologist to keep track of which cells have been counted already. Nuclei with weak to strong diffuse labeling and nuclei with only nucleolar labeling are counted. Avoid areas of ulceration and inflammation. For heavily pigmented neoplasms, bleaching the sections AFTER immunohistochemical labeling may be needed to better assess the Ki67 index. By assessing the Ki67 index as a percentage of positive nuclei in 500 cells, the number of cells evaluated is standardized. It is also much easier to identify the areas with the most proliferation by looking for red nuclear labeling than it is to identify an area of high mitotic activity when scanning a tumor. Thus, this marker is more objective than mitotic count. A threshold value of 15% has been empirically determined and has been evaluated in regard to survival with Kaplan-Meier survival curves.^{Laprie} In one study,

none of the behaviorally benign cutaneous melanocytic neoplasms had a Ki67 index greater than or equal to 15%.^{Laprie} Statistically significant lower survival rates were reported for dogs with neoplasms with a Ki67 index \geq 15%.^{Lacroux,Laprie} The percentage of correctly classified neoplasms using the Ki67 index (97%) was higher than that of mitotic count (91%) and histological criteria (93%) in one study.^{Laprie} Thus, a threshold of 15% should be used to predict prognosis of cutaneous melanocytic neoplasms.^{Smedley 2011b, Lacroux,Laprie}

Ideally, using the criteria above, a cutaneous melanocytic neoplasm should be able to be categorized as a cutaneous melanocytoma (benign melanocytic tumor) or a cutaneous malignant melanoma. Typically, a cutaneous melanocytoma is non-ulcerated, often raised, generally small (<2 cm diameter and \leq 0.45 cm thickness), limited to the dermis, heavily pigmented, has very bland appearing nuclei, and has a low mitotic count and very low Ki67 index.^{Lacroux, Spangler, Laprie, Silvestri} In some cases, a cutaneous malignant melanoma may have several of the same features as a melanocytoma but may have only one or two parameters that suggest malignant behavior, such as high nuclear atypia, a mitotic count above the threshold value, a tumor thickness > 0.95cm, extension beyond the dermis, or a Ki67 index above the threshold value. When there are mixed results, results for each parameter should be reported, but Ki67 index should be used for final interpretation in most cases. In cases that are still ambiguous, a diagnosis of melanocytic neoplasm should be made and each parameter should be discussed. Clear-cut cutaneous malignant melanomas are often ulcerated, poorly pigmented, exhibit marked nuclear atypia, have a high mitotic count and a high Ki67 index, are >0.95 cm thick, extend beyond the dermis, and may exhibit vascular invasion.^{Lacroux,Spangler,Silvestri}

A few other studies have evaluated various molecular markers as potential prognostic markers for cutaneous melanocytic neoplasms and some may hold future promise. However, most have low sample sizes, limited or no follow-up, or other limitations that prevent them from being used in a diagnostic setting at this time. One such marker that requires further investigation is survivin. In one study, nuclear survivin expression was significantly greater in malignant melanomas compared to melanocytomas, and increased expression (\geq 8% of neoplastic cells) was related to the presence of metastasis and death of the animal due to melanoma.^{Bongiovanni}

RACK1 is another marker that has been investigated as a potential diagnostic, as well as a potential prognostic, marker.^{Campagne} In one study, RACK1 distribution differed between benign and malignant canine melanocytic neoplasms and a RACK1 homogeneous labeling pattern was highly correlated with other criteria such as classic histologic features of malignancy, Ki-67 index, and mitotic count.^{Campagne} However, sample size was very small and included both cutaneous and mucosal neoplasms, and there was a lack of follow-up data in this study; thus, additional testing is needed before this marker can be used in a diagnostic setting. Even then, evaluation of this marker in a diagnostic setting may prove to be too complicated and it has not been shown to add any additional value over Ki67 index and mitotic count. In addition, other neoplasms can also label for RACK 1, so this marker cannot be used as a standalone marker for diagnosis of melanocytic origin.

FoxP3 and IDO expression have also been shown to hold promise as prognostic markers for canine cutaneous and oral melanocytic neoplasms, but also require further evaluation with larger sample sizes, with comparisons to other established prognostic markers, and by separating cutaneous from oral melanomas.^{Porcellato} Ideally, this should be done with a

prospective study. In addition, the methods used to evaluate FoxP3 and IDO expression in that study seem somewhat cumbersome to utilize in a diagnostic setting.

A few studies have evaluated the role of *c-Kit* in canine cutaneous, as well as oral, melanocytic neoplasms but no significant association between KIT labeling and survival time has been demonstrated.^{Gomes, Murakami, Gramer, Simpson} Gomes et al. demonstrated decreased expression of KIT protein in cutaneous malignant melanomas compared to cutaneous melanocytomas.^{Gomes} The decreased number of cells labeled in malignant versus benign tumors may give some insight into the role of *c-Kit* in melanocytic tumor progression, but the lack of correlation of either labeling extension or intensity with other morphologic grading factors does not suggest this will be an important prognostic marker or diagnostic tool. The Gramer et al. study only included three melanomas and two of the three showed KIT expression.^{Gramer} One of these tumors had a missense, or non-synonymous, mutation, but this was not a prognostic study and the sample size was too small to draw any significant conclusions.^{Gramer}

Other studies that included canine cutaneous melanocytic neoplasms could not demonstrate prognostic utility for the markers that they evaluated, were too preliminary to make any significant conclusions, were not designed as prognostic studies, or only used melanoma cell lines.^{Grandi, Han JI 2013, Pires, Seo, Finotello, Gillard, Abou} These markers included: S100A4 immunoexpression; E-cadherin/ β -catenin expression; COX-1 and COX-2 expression; P-glycoprotein 1 expression; and expression of MCAM/CD146.^{Grandi, Han JI 2013, Pires, Seo, Finotello, Abou}

Canine Oral/lip Mucosal Melanocytic Neoplasms

Histologic criteria can be used to identify two main subtypes of canine oral/lip melanocytic neoplasms, one being oral malignant melanomas and the other being histologically well-differentiated oral/lip melanocytic neoplasms (HWDM),^{Esplin} which are also known as canine oral melanocytic neoplasms of low malignant potential.^{Simpson}

Mitotic count, nuclear atypia, degree of pigmentation, level of invasion, and vascular invasion are the primary histologic features used for prognostication of oral/lip melanocytic neoplasms in dogs.^{Smedley 2011b, Spangler, Esplin, Bergin} Again, while mitotic count is more objective than nuclear atypia, it is still subject to significant inter- and intraobserver variation. However, it has shown statistical relevance in several studies of canine oral/lip melanocytic neoplasms.^{Smedley 2011b, Spangler, Bergin} Mitotic count should be determined in the area of highest mitotic activity.^{Smedley 2011b, Bergin} Therefore, first scan the neoplasm at 100x magnification/10x objective to locate areas of potentially high mitotic activity. Locate a field containing one or more mitotic figures, if possible, and begin counting mitoses in that area at 400x magnification/40x objective (hpf). Count mitotic figures in 10 consecutive hpf. Avoid areas of ulceration, necrosis and inflammation when counting mitoses. As mentioned above, the size of the ocular used should also be reported, in order to improve the ability to compare mitotic counts between different studies.^{Meuten} Ideally, mitotic counts should now be reported per 2.37mm², which can be obtained by counting mitoses in 10 hpf with an FN22 ocular. For heavily pigmented neoplasms bleaching may be needed to better assess the mitotic count. A cut-off of 4 mitoses per 10 hpf is a statistically determined threshold value for mitotic count.^{Bergin} A marked difference in survival between the two groups created by this cut-off value has been demonstrated with a sensitivity of 90% and a specificity of 84% in one study.^{Bergin}

Similar to cutaneous melanocytic neoplasms, nuclear atypia should be assessed according to the criteria outlined by Spangler and Kass (2006)^{Spangler} in order to decrease the potential for interobserver variation. These criteria are repeated here: Well-differentiated nuclei are small with a single centrally located nucleolus and minimal clumping of chromatin. They may have condensed strands of nuclear chromatin extending from the nucleolus to the nuclear membrane or condensation of chromatin along the inner surface of the membrane. Cells that lack a nucleolus have fine and evenly dispersed chromatin at the periphery of the nucleus. Poorly differentiated nuclei have larger nucleoli of less regular shape that are eccentrically located within the nucleus. There are often multiple nucleoli that, in some cases, may be haphazardly connected to the inner surface of the nuclear membrane by thin strands of chromatin and give the appearance of a coarsely vacuolated nucleus.^{Spangler} Again, the nuclear criteria may be very difficult, or not possible, to assess in some spindle, whorled/dendritic, or balloon/clear/signet ring cell variants of melanocytic neoplasms.^{Spangler} Using the above criteria for well-differentiated versus poorly differentiated nuclei, oral and lip melanocytic neoplasms should subjectively be determined to contain less than 30% atypical nuclei or greater than or equal to 30% atypical nuclei.^{Smedley 2011b,Spangler,Bergin} The most accurate way to do this is to count the number of atypical nuclei among 200 cells. This threshold value for semi-quantification has been shown to have statistical significance for predicting survival times based on Kaplan-Meier survival curves.^{Bergin} For heavily pigmented neoplasms bleaching may be needed to better assess nuclear features.

While pigmentation is difficult to measure objectively with validated cutoff points, a high degree of pigmentation does suggest a favorable clinical outcome for oral/lip melanocytic neoplasms.^{Smedley 2011b,Bergin} Neoplasms with $\geq 50\%$ of pigmented cells have been shown to have longer survival times.^{Smedley 2011b,Bergin} However, outcome is not predictable in oral/lip neoplasms with moderate, low, or no pigmentation.^{Smedley 2011b,Bergin} Pigmentation should be evaluated but should not be used as a sole predicting factor. In addition, lack of bone lysis on skull radiographs has been recommended as a valid predictor of longer survival times in dogs with oral malignant melanomas.^{Smedley 2011b}

As with cutaneous melanocytic neoplasms, more than one histologic parameter should be used to classify a melanocytic neoplasm and when they conflict with one another, both the favorable and poor prognostic factors should be discussed. When an oral/lip melanocytic neoplasm has marked nuclear atypia ($\geq 30\%$ atypical nuclei), a high mitotic count ($\geq 4/10$ hpf), or evidence of vascular invasion or metastasis, the neoplasm should be diagnosed as a malignant melanoma and evaluation of the Ki67 index should be offered for further prognostication. Pathologists may indicate in a report that Ki67 index is not necessary for neoplasms that greatly surpass the nuclear atypia and mitotic count thresholds and/or show evidence of vascular invasion or metastasis. There are no established values for “greatly surpassing” the cut-offs, but pathologists may use their experience to subjectively make this recommendation. In general, the committee agrees that Ki67 index is not necessary for tumors with $\geq 50\%$ atypical nuclei or a mitotic count $\geq 40/10$ hpf, which is 10 times higher than the threshold value. When these features are lacking, nuclear atypia and mitotic count are near the established threshold values for malignancy, or if nuclear atypia and mitotic count are disparate, evaluation of the Ki67 index should be performed.^{Smedley 2011b}

Some oral/lip melanocytic neoplasms with histological criteria of malignancy, but low Ki67 index have been shown to have longer survival times than expected by histology.^{Esplin,Bergin} **Thus, it is never wrong to perform Ki67 index.** In one study, the positive predictive value with respect to outcome at one year was 86.3% when classified by the Ki67 threshold, 82.5% when classified by nuclear atypia, and 79% when classified by mitotic count.^{Bergin} The Ki67 index is determined as the average number of positively labeled neoplastic cell nuclei per area of a 1cm² optical grid reticle at 400x magnification/40x objective (5 grid areas counted) in the highest labeling area.^{Bergin} Nuclei with weak to strong diffuse labeling and nuclei with only nucleolar labeling are counted. Avoid areas of ulceration and inflammation. For heavily pigmented neoplasms, bleaching the sections AFTER immunohistochemical labeling may be needed to better assess the Ki67 index. This grid method standardizes the area assessed so that it is the same no matter what microscope is used. It is also much easier to identify the areas with the most proliferation by looking for red nuclear labeling than it is to identify an area of high mitotic activity when scanning a tumor. A threshold value of 19.5 was statistically determined using a receiver operator curve.^{Bergin} Kaplan-Meier survival analysis showed that the survival curves for dogs with a Ki67 index < 19.5 and dogs with a Ki67 index ≥ 19.5 are significantly different based on a one-year survival period (P<.0001).^{Bergin}

Ideally, using the criteria above, a mucosal lip or oral neoplasm should be able to be categorized as an oral malignant melanoma or a histologically well-differentiated melanocytic neoplasm (HWDM),^{Esplin} also known as a melanocytic neoplasm of low malignant potential.^{Simpson} The term melanocytoma should be avoided for oral and lip mucosal melanocytic neoplasms as the long term (>2 years) behavior of these neoplasms, if not excised, is still somewhat uncertain. Thus, they should be treated as neoplasms of low malignant potential. HWDMs are usually raised, non-ulcerated, <2cm diameter, heavily pigmented, lack cellular atypia, do not invade bone, and have rare mitoses, a very low Ki67 index, and abundant collagenous stroma.^{Esplin,Simpson} They also often lack junctional activity and lateral surface epithelial spread which improves the chance of complete excision and likely plays a role in the longer survival time of these tumors.^{Simpson} A mean survival time of 23.4 months has been reported for HWDMs in one study.^{Esplin} These features are in strict contrast to oral malignant melanomas which often show junctional activity, lateral epithelial spread, poor pigmentation, bone invasion, and marked nuclear atypia and often have a very high mitotic count and Ki67 index.^{Spangler,Bergin} When there are mixed results, results for each parameter should be reported, but Ki67 index should be used for final interpretation in most cases.^{Smedley 2011b} In cases that are still ambiguous, a diagnosis of melanocytic neoplasm should be made and each parameter should be discussed.^{Smedley 2011b}

There are a few new prognostic markers that have been investigated for canine oral melanocytic neoplasms since 2011 and a few of them show potential usefulness. For example, lussich *et al.*^{lussich} provides good statistical support for considering platelet-derived growth factors receptors (PDGFR)- α and - β as prognostic markers in oral malignant melanoma of dogs. The purpose of this study was to evaluate the expression of PDGFR- α and - β in stage II and III canine oral malignant melanomas and to correlate it with prognosis. The neoplasms in this study were confirmed as melanocytic in origin via IHC labeling for PNL-2 and pathologists recorded nuclear atypia, mitotic count, pigmentation, and Ki67 values for each tumor.^{lussich} This study suggests that PDGFRs may play a role in the pathogenesis of oral canine malignant

melanoma and the co-expression of both PDGFRs- α and - β should be considered as a negative prognostic marker. In addition, despite an unclear methodology for determining Ki67 index that is not consistent with the published method by Bergin et al. (2011),^{Bergin} statistical analysis showed that co-expression of PDGFRs and Ki67 values were both associated with worse prognosis, further supporting the prognostic importance of Ki67 index. PDGFRs were detected not only in tumor tissue but also in the stroma, suggesting a potential role in matrix remodeling and tumor invasion.^{lussich} The authors stated that further prospective studies on a greater number of cases are warranted to confirm these findings before they are used in a diagnostic setting.^{lussich}

RACK1, FoxP3, and IDO are additional markers that have been examined in both cutaneous and oral melanocytic neoplasms and may hold future promise. These markers were discussed above under cutaneous melanocytic neoplasms. Also discussed above under cutaneous melanocytic neoplasms was the role of *c-Kit*. Murakami et al. evaluated KIT labeling in canine oral malignant melanomas and found no association with overall survival or WHO stage.^{Murakami} In addition, no significant mutations were identified in exon 11 of *c-Kit*.^{Murakami} While this study did have some limitations, such as relatively low sample size, lack of complete detail regarding diagnosis and follow-up times, and the inclusion of only malignant melanomas, this study concluded that KIT expression does not appear to be a prognostic factor for canine melanoma and there is no indication of a mutation to suggest tyrosine kinase inhibitors would be beneficial.^{Murakami}

Other studies that were reviewed and included oral melanocytic neoplasms could not demonstrate prognostic utility for the markers that they evaluated, were too preliminary to make any significant conclusions, were not designed as prognostic studies, or only used melanoma cell lines.^{Kawabe, Han JI 2013, Pires, Seo, Finotello, Gillard, Abou, Fowles, Teixeira, Jama} These markers included: a metabolite profile that included citric acid, lactic acid, oleic acid, linoleic acid, palmitoleic acid, octadecenoic acid, and glycerol; E-cadherin/ β -catenin expression; COX-1 and COX-2 expression; P-glycoprotein 1 expression; expression of MCAM/CD146; activation of the MAPK and PI3K/AKT pathways; the expression pattern of Cx26 and Cx43; and nBAP1 protein expression.^{Kawabe, Han JI 2013, Pires, Seo, Finotello, Abou, Fowles, Teixeira, Jama}

Future directions / Recommended studies -

None of the published prognostic studies meet all of the standards defined by the Webster et al. (2011) white paper but the recommendations in this consensus are based on the results of the studies that adhere to most of those standards. The first step in any prognostic study is to ensure an accurate diagnosis of melanocytic origin for each case in the study population. This cannot be achieved by using single non-specific IHC markers such as S-100. Once melanocytic origin is established, neoplasms should be classified based on location as well as established morphologic and molecular prognostic criteria such as nuclear atypia, mitotic count, and Ki67 index. Another major requirement for a sound prognostic study is follow-up data that includes disease free survival times, progression free survival times, overall survival, cause of death, and, ideally, post-mortem results. Collection of follow-up data is one of the most challenging tasks in veterinary medical research studies and post-mortem examination of

a significant number of cases is often not possible. However, researchers should always have an initial study design that will allow for as much follow-up data as possible.

There are very few published studies that are true prognostic studies of canine melanocytic neoplasms and only one of these evaluated prognostic studies^{Teixeira} is prospective. While prospective studies are very challenging studies to conduct in veterinary medicine, ideally an effort should be made to conduct such studies in order to verify the recommendations made by the referenced retrospective studies. Additionally, new potential prognostic markers should be evaluated in conjunction with the current statistically proven prognostic parameters, such as nuclear atypia, mitotic count, and Ki67 index.

Much of the recent literature has focused on the genetic features of canine melanocytic neoplasms, and this field holds promise for better prognostication and prediction of metastasis of these tumors, as well as for identification of potential targets for therapy. In the future, genetic analysis may prove to be the gold standard for prognostication but, at this point, the most accurate predictors of prognosis we have include the histologic features described above and the Ki67 index.

Additionally, a future consensus that evaluates clinical prognostic parameters is planned. It is hoped that a combination of histologic, molecular, and clinical parameters will further improve our ability to prognosticate these neoplasms, in order to better determine appropriate therapy for each case.

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CRITICALLY REVIEWED LITERATURE

Smedley RC, Spangler WL, Esplin DG, Kitchell BE, Bergman PJ, Ho H, Bergin IL, Kiupel M: Prognostic markers for canine melanocytic neoplasms: a comparative review of the literature and goals for future investigation. *Vet Pathol* 2011; 48:54-72

Objectives:

To conduct a detailed literature review of canine melanocytic neoplasia publications prior to February 2011 and evaluate the different postulated prognostic classification schemes according to the published Recommended Guidelines for the Conduct and Evaluation of Prognostic Studies in Veterinary Oncology.

Study Design:

Review paper

SUBGROUP CONCLUSIONS:

The paper is a review of the literature prior to February 2011 with the ultimate conclusion: The percentage of correctly classified canine melanocytic neoplasms and the accurate prediction of biological behavior can be maximized by applying numeric criteria to specific histological features that primarily include evaluation of nuclear atypia, mitotic count, Ki67 index, degree of pigmentation, and evidence of ulceration (cutaneous neoplasms only).

Przedziecki R, Czopowicz M, Sapierzynski R: Accuracy of routine cytology and immunocytochemistry in preoperative diagnosis of oral amelanotic melanomas in dogs. *Vet Clin Pathol* 44/4 (2015) 597–604

Objectives:

Data on diagnostic performance of ICC in differential diagnosis of canine oral cancers in veterinary practice are lacking. Therefore, the aim of this study was to assess the accuracy of routine cytology in the diagnostic workup of oral canine amelanotic melanoma, and to demonstrate the usefulness of ICC in precise preoperative cytologic diagnosis of these canine malignancies.

Study Design:

Prospective

SUBGROUP CONCLUSIONS:

This study did not aim to evaluate prognostic markers. Instead, it had a diagnostic purpose: to assess the reliability of routine cytology and immunocytochemistry in preoperative diagnosis of canine oral amelanotic melanoma. Poor pigmentation is common in oral melanomas and the authors found that immunocytochemistry improved the ability to diagnose these tumors by cytology. In this small study, the diagnosis reached with immunocytochemistry matched the histologic diagnosis. A more thorough description of the intensity and expression of the labeling would be helpful. There was no mention of atypia or other H & E characteristics.

Silvestri S, Porcellato I, Mechelli L, Menchetti L, Rapastella S, Brachelente C: Tumor Thickness and Modified Clark Level in Canine Cutaneous Melanocytic Tumors. Vet Pathol. 2019 Mar;56(2):180-188. doi: 10.1177/0300985818798094. Epub 2018 Sep 24. PMID:30244658

Objectives:

The aim of the study was to examine the applicability and usefulness of assessing tumor thickness and the Clark level in canine cutaneous melanocytic tumors and their association with survival and hazard of death. They evaluated a more convenient system for measuring the tumor thickness that can be adopted in any laboratory, since not all laboratories have an ocular micrometer for measurements.

Study Design:

Retrospective

SUBGROUP CONCLUSIONS:

The subgroup agrees with the authors' conclusions. This was a well-designed study with thorough statistics. While the prognostic significance of tumor thickness could not be confirmed with multivariable analysis, univariate analysis did support this parameter as a useful prognostic marker that could be combined with other prognostic markers and the survival curves were solid. Statistically determined thresholds were identified for tumor thickness and the subgroup recommends reporting this parameter for diagnostic cases. This study also further confirmed the usefulness of mitotic index, nuclear atypia, degree of pigmentation and ulceration as prognostic markers for cutaneous melanocytic neoplasms.

Lacroux C, Raymond-Letron I, Bourges-Abella N, Lucas MN, Deviers A, Serra F, Degorce-Rubiales F, Delverdier M. Study of canine cutaneous melanocytic tumours: evaluation of histological and immunohistochemical prognostic criteria in 65 cases. 2012 *Revue Méd. Vét.*, 163, 8-9, 393-401.

Objectives:

The 2 main objectives of this study were to evaluate the prognostic value of histocytopathological features of the neoplasms as well as Melan A/MART-1 and CD44 expression by neoplastic cells, in comparison with Ki-67 expression.

Study Design:

Retrospective

SUBGROUP CONCLUSIONS:

This study further confirmed the usefulness of mitotic index, lymphatic or vascular invasion, and Ki67 labeling as excellent prognostic indicators for cutaneous melanocytic tumors. It also supported the use of tumor thickness, growth pattern, and symmetry of the neoplasm for predicting prognosis. These additional features should be considered for prognostication of cutaneous melanocytic neoplasms. In addition, the authors evaluated CD44 expression as a potential prognostic marker. This marker may be combined with Ki67 index to improve prognostication but additional studies are needed to confirm these findings before put into regular use for diagnostics.

Iussich S, Maniscalco L, DiSciuva A, Iotti B, Morello E, Martano M, Gattino F, Buracco P, and DeMaria R: PDGFRs expression in dogs affected by malignant oral melanomas: correlation with prognosis. Vet Comp Oncol. 2017 Jun;15(2):462-469. doi: 10.1111/vco.12190.

Objectives:

The purpose of this study was to evaluate the expression of platelet-derived growth factors receptors (PDGFR)- α and - β in stage II and III canine oral malignant melanomas and to correlate it with prognosis.

Study Design:

Retrospective

SUBGROUP CONCLUSIONS:

The results of this study provide solid statistical support for considering PDGF-alpha and -beta as prognostic markers in oral melanoma of dogs. The study also supports the prognostic importance of Ki67, despite a slightly different method for determining Ki67 index and an unclear methodology for this determination. Free versus infiltrated surgical margins (CMM considered inoperable, i.e. with no chance to get clean margins at surgery, were not included here) and clinical stage failed to correlate with survival. Further prospective studies on a greater number of cases are warranted to confirm this finding.

Campagne C, Julé S, Alleaume C, Bernex F, Ezagal J, Château-Joubert S, Estrada M, Aubin-Houzelstein G, Panthier JJ, Egidy G. Canine melanoma diagnosis: RACK1 as a potential biological marker. Vet Pathol. 2013; Nov;50(6):1083-90.

Objectives:

The aim of this study was to assess the value of RACK1 detection by immunofluorescence and immunohistochemistry in canine melanoma diagnosis. The authors also aimed to correlate RACK1 distribution with prognostic markers of tumoral samples.

Study Design:

Retrospective

SUBGROUP CONCLUSIONS:

The results of this study suggest a new potential prognostic marker, RACK 1, for canine melanocytic tumors, as the labelling pattern for RACK1 showed high concordance with malignancy criteria (histology, Ki67, mitotic count, other features of malignancy). However, the limited number of cases analyzed, and the lack of follow up data, represent the two main limitations of the study. It also needs to be evaluated in conjunction with the labeling of neoplastic melanocytes with a specific melanocytic marker as other types of neoplasms can label with this marker. This may be somewhat complicated in a routine diagnostic setting. The sample sizes from which they drew conclusions about lip and digit melanomas are too small to be clinically relevant. In addition, this study included melanocytic neoplasms from many different locations (cutaneous and mucosal) which confounds the data. At this point, this marker has not been shown to add any additional value over Ki67 index and mitotic count. In order to determine the true prognostic value of RACK1, RACK1 labeling should be performed on a group of tumors with known outcome, or, ideally, in a prospective manner.

Bongiovanni L, D'Andrea A, Porcellato I, Ciccarelli A, Malatesta D, Romanucci M, Della Salda L, Mechelli L, Brachelente C: Canine cutaneous melanocytic tumours: significance of β -catenin and survivin immunohistochemical expression. *Vet Dermatol.* 2015 Aug;26(4):270-e59. doi: 10.1111/vde.12211

Objectives:

The aim was to investigate the immunohistochemical expression of survivin and β -catenin in 21 canine cutaneous melanocytic tumors, and to investigate the possible association of their expression patterns with histological features and malignant behavior, in order to understand their prognostic significance (histologic features of malignancy, metastatic disease and melanoma-related death.)

Study Design:

Retrospective

SUBGROUP CONCLUSIONS:

Based on comparison of results of this study to results in previous studies as described in the discussion, survivin seems to be the marker with the most promise as a prognostic factor.

Some concerns are the relatively small sample size, there were few cases with clinical follow-up, the lack of details concerning the diagnosis of the metastatic lesions, the lack of information concerning any adjunct therapies that may have been used, and the details of the cause of death in those dogs that died. It was also unclear what was meant by “tumor cells near vessels”. If metastasis was confirmed histologically in the four cases, it does appear those cases had a higher level of expression of both markers and they could be useful if the findings are confirmed statistically in a larger number of cases. It would be nice to compare survivin expression with Ki67 index.

The study determined the designation of malignancy based on the mitotic count, cellular/nuclear pleomorphism, ulceration and infiltrative growth. Mitoses were evaluated by counting the number of mitotic figures in 10 random, non-overlapping fields at the highest magnification (HPF, 40X) in H&E-stained slides. A mitotic count of $\geq 3/10$ HPF was considered as the main parameter for the histological classification of (malignant) melanomas. However, when comparing melanocytomas to melanomas, a relationship was observed between malignancy and the presence of ulceration, necrosis and atypical mitotic figures ($P < 0.05$), as well as cellular pleomorphism, number of mitoses and the presence of neoplastic cells close to the vessels ($P < 0.01$). It is unclear how the same histologic features that were used to make a histologic diagnosis of a malignant melanoma could then be associated with malignancy, unless this related to behavior, which is not stated.

Porcellato I, Brachelente C, De Paolis L, Menchetti L, Silvestri S, Sforza M, Vichi G, Iussich S, Mechelli L: FoxP3 and IDO in Canine Melanocytic Tumors. Vet Pathol. 2019 Mar;56(2):189-199. doi: 10.1177/0300985818808530

Objectives:

The aim of this study was to provide preliminary data on the expression of transcription factor forkhead box protein P3 (FoxP3) and indoleamine 2,3-dioxygenase (IDO) in primary canine melanocytic tumors and to investigate their prognostic role.

Study Design:

Retrospective

SUBGROUP CONCLUSIONS:

The subgroup concurs with the authors' conclusions above. The was a well-designed study with sound statistics and well-established cut-off values. The limitations discussed by the authors warrant further studies with larger sample sizes and with comparison of these markers to other established canine melanocytic tumor prognostic markers in order to confirm the prognostic value of FOXP3 and IDO. Ideally, this should be done with a prospective study. In addition, the methods of determining FOXP3+ cells/HPF, %FoxP3, and IDO+ cells/HPF seem somewhat cumbersome to utilize in a diagnostic setting.

Kawabe M, Baba Y, Tamai R, Yamamoto R, Komori M, Mori T, Takenaka S.: Profiling of plasma metabolites in canine oral melanoma using gas chromatography-mass spectrometry. J Vet Med Sci. 2015 Aug; 77(8): 1025–1028

Objectives:

To examine metabolite profiles in plasma from dogs with oral malignant melanoma using gas chromatography-mass spectrometry (GC-MS) with the goal of identifying biomarkers for diagnosis of the tumor and prediction of prognosis.

Study Design:

Prospective

SUBGROUP CONCLUSIONS:

This small study begins to shed light on a variety of metabolites that vary between normal canine patients and those with melanoma. However, a greater population size and correlation with other disease processes is necessary to determine the specificity of these metabolites. Samples were only taken after radiation therapy. It is not known how radiation may change these results as there were no before and after samples to compare.

Teixeira TF, Gentile LB, da Silva TC, Menecier G, Chaible LM, Cogliati B, Roman MA, Gioso MA, Dagli ML: Cell proliferation and expression of connexins differ in melanotic and amelanotic canine oral melanomas. Vet Res Commun 2014; 38: 29
<https://doi.org/10.1007/s11259-013-9580-z>

Objectives:

To determine whether oral amelanotic melanomas behave differently from melanotic melanomas in dogs, relative to aggressiveness and survival time after diagnosis. Cell proliferation was quantified in these two melanoma types via mitotic count and measurement of PCNA. The expression of the gap junction proteins, connexins 26 and 43, were also evaluated.

Study Design:

Prospective

SUBGROUP CONCLUSIONS:

Within this study population, canine amelanotic melanomas presented more aggressive behavior when compared to melanotic counterparts; however, the patient cohort was small albeit uniform regarding oral location. These results do support findings in previous studies that have shown that heavily pigmented melanocytic neoplasms (those with greater than 50% pigmentation) have a more favorable outcome. Those previous studies however, could not necessarily demonstrate a worse prognosis for neoplasms with low pigmentation. In the current study, there appears to be no distinction between dogs that had surgery or not and there is no discussion of surgical outcome (ie. whether clean margins were obtained at

surgery); thus, we question the time to progression data. Likewise, there is no discussion of where the 5 dogs with metastatic lesions fit into the time to progression data. Finally, there is no discussion of location in the oral cavity.

Limitations in study design (small sample size, short follow-up interval, uncertainty of consistent treatment) compromise the authors' conclusions. At this point, there is no evidence to support using Connexin expression for prognostication of canine oral melanomas.

Grandi F, Rocha RM, Miot HA, Cogliati B, Rocha NS: Immunoexpression of S100A4 in canine skin melanomas and correlation with histopathological parameters. Vet Q. 2014; 34(2):98-104 doi: 10.1080/01652176.2014.936628

Objectives:

To retrospectively verify S100A4 immunoexpression in tumor stroma and neoplastic cells and determine whether it correlates with histopathological parameters in dogs. (S100A4 is considered a marker with high prognostic significance in humans, functioning primarily in tumor progression, cancer cell motility, metastasis, invasiveness, apoptosis, tumor angiogenesis, upregulation of matrix metalloproteinases, and high-grade histomorphology.)

Study Design:

Retrospective

SUBGROUP CONCLUSIONS:

The results of this study showed that S100A4 is expressed in tumor stroma and neoplastic melanocytes. However, the authors could not show an association with survival or presence/absence of metastasis due to the lack of follow-up information. The authors decided to apply Clark's levels of invasion try to correlate S100A4 expression with the Clark's levels of invasion. However, most canine melanomas are primarily dermal and Clark's system is not typically used in veterinary medicine since its prognostic significance has not definitively been proven. Also, whether adjunct therapy was applied to the patients in this study was not indicated.

Han JI, Kim Y, Kim DY, Na KJ: Alteration in E-cadherin/ β -catenin expression in canine melanotic tumors. Vet Pathol. 2013 Mar;50(2):274-80. doi: 10.1177/0300985812457792

Objectives:

Aims of this study: (1) to examine the dysregulation of β -catenin in canine oral melanoma, (2) to examine correlations of E-cadherin/ β -catenin complex levels with the mutational status of β -catenin exon 3, and (3) to identify any correlation between skin and oral melanotic tumors. The Authors proposed that the dysregulation of β -catenin also occurs in canine oral melanoma.

Study Design:

Retrospective

SUBGROUP CONCLUSIONS:

Small number of samples assessed limits the usefulness of this study. From the results showed in the present work, the *ctnnb1* mRNA expression levels, nor the beta-catenin and E-cadherin membranous and cytoplasmic expression, nor the beta-catenin nuclear localization appeared to be useful prognostic markers. Their altered expression are reported in both skin and oral, benign and malignant melanoma, and they seem to be related more in melanocytic tumor development rather than in their progression. While compelling as a mechanism that is worthy of further study, E-cadherin/ β -catenin expression is not yet useful for diagnosis or prognosis.

Pires I, Garcia A, Prada J, Queiroga: FLCOX-1 and COX-2 expression in canine cutaneous, oral and ocular melanocytic tumours. J Comp Pathol. 2010 Aug-Oct;143(2-3):142-9. doi: 10.1016/j.jcpa.2010.01.016.

Objectives:

In order to evaluate the potential value of non-steroidal anti-inflammatory drugs (NSAIDs) in the treatment of canine malignant melanoma, expression of cyclooxygenase (COX)-1 and COX-2 was determined in 20 cutaneous, nine oral and two ocular malignant melanomas, and in nine cutaneous melanocytomas. The aim of the present study was to examine COX-1 and COX-2 expression in different histological types of canine melanocytic tumors.

Study Design:

Retrospective

SUBGROUP CONCLUSIONS:

COX-2 expression may have prognostic significance in canine melanomas but this needs to be evaluated in conjunction with survival data, tumor progression, metastasis, etc., ideally in a study population with uniform therapy. Evaluation in association with mitotic count, nuclear atypia, and Ki67 index would also be important.

Seo KW, Coh YR, Rebhun RB, Ahn JO, Han SM, Lee HW, Youn HY: Antitumor effects of celecoxib in COX-2 expressing and non-expressing canine melanoma cell lines. Res Vet Sci 2014; 96:482-486

Objectives:

The purpose of this study was to investigate the anticancer effects of celecoxib on canine malignant melanoma cell lines that express varying levels of COX-2. To expose two canine melanoma cells lines, one cutaneous and not expressing COX-2 and one oral that expressed COX-2, to a COX-2 inhibitor and then measure prostaglandin E2 expression, cell cycle and apoptosis in each cell line.

Study Design:

Investigative

SUBGROUP CONCLUSIONS:

This is not a prognostic study. It appears there is evidence that COX-2 inhibitors have the potential for antitumor effects in melanomas in vitro.

Finotello R¹, Monné Rodriguez JM², Vilafranca M³, Altimira J³, Ramirez GA³, Haines A⁴, Ressel L: Immunohistochemical expression of MDR1-Pgp 170 in canine cutaneous and oral melanomas: pattern of expression and association with tumour location and phenotype. Vet Comp Oncol. 2017 Dec;15(4):1393-1402. doi: 10.1111/vco.12281

Objectives:

The authors hypothesized that Pgp (P-glycoprotein 1 (Pgp) expression is a well-recognized feature of multi-drug resistance that could play a role in canine malignant melanoma (CMM), conferring an intrinsic MDR phenotype; therefore, the aim of this study was to investigate Pgp immunoreactivity in treatment-naïve oral and cutaneous CMM and to describe its pattern of expression and its association with tumor location and histological phenotype.

Study Design:

Retrospective

SUBGROUP CONCLUSIONS:

This is not a prognostic study. Only malignant melanomas were included, 12 oral and 13 cutaneous. The Ki67 index was not determined. Mitotic counts were determined. There was only limited survival/progression data. At this point, there is no support to use Pgp as a prognostic marker in dogs with melanocytic tumors.

Gomes J, Queiroga FL, Prada J, Pires I. Study of c-kit immunoexpression in canine cutaneous melanocytic tumors. Melanoma Res. 2012 Jun;22(3):195-201

Objectives:

The aims of this study are to analyze c-kit expression in canine cutaneous melanocytic tumors and associate it with tumor behavior (benign or malignant)/progression, in order to investigate the dog's potential in comparative pathology and c-kit's potential in the diagnosis of these tumors.

Study Design:

Retrospective

SUBGROUP CONCLUSIONS:

The decreased number of cells labeled in malignant vs. benign tumors may give some insight into the role of c-kit in melanocytic tumor progression, but the lack of correlation of either

labeling extension or intensity with other morphologic grading factors does not suggest this will be an important prognostic marker or diagnostic tool. Seems that decreased KIT expression is more common in malignant tumors, but not found in all.

Murakami A, Mori T, Sakai H, Murakami M, Yanai T, Hoshino Y, Maruo K: Analysis of KIT expression and KIT exon 11 mutations in canine oral malignant melanomas. Vet Comp Oncol. 2011 Sep;9(3):219-24. doi: 10.1111/j.1476-5829.2010.00253.x.

Objectives:

To evaluate the expression of c-kit in canine oral malignant melanomas, to check for mutations in exon 11 and to evaluate for an association between c-kit expression and histologic features and prognosis.

Study Design:

Retrospective

SUBGROUP CONCLUSIONS:

There was a relatively small case number. The study lacks details concerning tumor diagnosis, therapies used, survival data including cause of death and definition of immunoreactivity intensity. A greater number of cells in OMM labeled for KIT than in other studies. Only malignant neoplasms were evaluated; there was no comparison to benign melanocytic neoplasms or those of low malignant potential. The authors state that KIT labeling is not prognostic and no exon 11 mutations were found.

Gramer I, Kessler M, Geyer J. Detection of novel polymorphisms in the c-kit gene of canine lymphoma, melanoma, hemangiosarcoma and osteosarcoma. Vet Res Commun 2016; 40:89-95

Objectives:

Detect KIT expression in the selected tumors; search for novel single nucleotide polymorphisms (SNPs) in cKIT hot spot regions for gain-of-function mutations and correlation of the identified cKIT polymorphisms with already known gain-of-function mutations.

Study Design:

Retrospective

SUBGROUP CONCLUSIONS:

Only 3 melanomas studied. One melanoma had a mutation that could suggest *c-Kit* is associated with tumor progression, but this is a preliminary study and more information is needed. In addition, as seen in the Gomes paper, there is some suggestion that *c-Kit* is expressed more in well differentiated melanocytes and may be reduced in malignant melanomas.

Jama N, Farquhar N, Butt Z, Coupland SE, Sacco JJ, Scase T, Fielding AB, Coulson JM, Kalirai H, Killick DR: Altered Nuclear Expression of the Deubiquitylase BAP1 Cannot be Used as a Prognostic Marker for Canine Melanoma. J Comp Pathol. 2018 Jul;162:50-58. doi: 10.1016/j.jcpa.2018.06.007. Epub 2018 Jul 18.

Objectives:

To characterize canine BAP1 and to compare nBAP1 protein expression in canine melanoma with that previously described in human melanoma. The authors hypothesized that loss of nBAP1 in canine oral and uveal melanoma would be associated with a poorer prognosis.

Study Design:

Retrospective

SUBGROUP CONCLUSIONS:

The subgroup concurs with the author's conclusions: BAP1 IHC cannot be used as a prognostic marker in canine uveal and mucosal melanoma.

Laver T, Feldhaeusser BR, Robot CS, Baez JL, Cronin KL, Buracco P, Annoni M, Regan RC, McMillan SK, Curran KM, Selmic LE, Shiu KB, Clark K, Fagan E, Thamm DH: Post-surgical outcome and prognostic factors in canine malignant melanomas of the haired skin: 87 cases (2003-2015). Can Vet J. 2018 Sep;59(9):981-987. PMID:30197441

Objectives:

Sought to examine clinical prognostic factors in a large group of cutaneous malignant melanomas treated with surgery to identify and update prognostic factors in this disease process.

Study Design:

Retrospective

SUBGROUP CONCLUSIONS:

The authors list a number of limitations of this study, and the group concurs. In addition, the method used to determine the mitotic count (mitoses in 10 hpf) for cases in this study is concerning. The authors' recorded the mitotic count from the original report so several different pathologists determined these counts. The authors did not recount mitoses. It was not stated whether the original pathologists had determined the number of mitoses by counting random fields or by counting in areas of highest mitotic activity. In addition, when mitotic counts were listed in the report as X number of mitoses per hpf, the authors multiplied this number by 10 and used that as the mitotic count for 10 hpf. This is a very inaccurate way of determining mitotic count, as mitoses are commonly irregularly distributed throughout a

neoplasm. Thus, there are no accurate conclusions that can be made relating to mitotic count in this paper.

Forty-eight patients received adjuvant therapy that included a mix of vaccine therapy, radiation therapy, and chemotherapy. Thus, therapy was not uniform in this group.

We do agree that the behavior of cutaneous malignant melanoma of the haired skin in dogs appears to take a predominantly benign course, and local control with surgery can lead to long survival times with dogs greater than 9.4 years having a greater risk for shorter OST and PST.

Gillard M, Cadieu E, De Brito C, Abadie J, Vergier B, Devauchelle P, Degorce F, Dréano S, Primot A, Dorso L, Lagadic M, Galibert F, Hédan B, Galibert MD, André C: Naturally occurring melanomas in dogs as models for non-UV pathways of human melanomas. Cell Melanoma Res. 2014 Jan;27(1):90-102. doi: 10.1111/pcmr.12170.

Objectives:

To define correspondences within the human histogenetic classification of melanoma with canine melanoma to propose the dog as a relevant model for the study of genes involving the non-UV dependent pathways of mucosal and acral melanomas.

Study Design:

Investigative

SUBGROUP CONCLUSIONS:

This is not a prognostic study.

Simpson RM¹, Bastian BC, Michael HT, Webster JD, Prasad ML, Conway CM, Prieto VM, Gary JM, Goldschmidt MH, Esplin DG, Smedley RC, Piris A, Meuten DJ, Kiupel M, Lee CC, Ward JM, Dwyer JE, Davis BJ, Anver MR, Molinolo AA, Hoover SB, Rodriguez-Canales J, Hewitt SM. Sporadic naturally occurring melanoma in dogs as a preclinical model for human melanoma. Pigment Cell Melanoma Res. 2014 Jan;27(1):37-47. doi: 10.1111/pcmr.12185

Objectives:

A series of oncogenic alterations in signaling components primarily of the MAP-kinase pathway have been identified. They affect genes such as BRAF, NRAS, KIT, HRAS, GNAQ, and GNA11 and, at least early in progression, are found in a mutually exclusive pattern. The individual mutations are associated with distinct clinical, histopathological, and epidemiological features in humans, suggesting that melanocytic neoplasia is comprised of biologically distinct subtypes. Genetic alterations in mucosal or oral sites have not been fully delineated in dogs.

This is a consensus paper comparing histopathologic features of human and canine melanoma specimens by MD and DVM pathologists working with basic science researchers. This group's

mission was to characterize and identify lines of future study to develop dogs as models for human clinical trials.

Study Design:

Retrospective and investigative

SUBGROUP CONCLUSIONS:

Follow-up data only available for 27 of 44 canine melanoma specimens. The purpose of this working group was to lay the groundwork for using dogs as preclinical models for human melanoma. This was not a true prognostic study but a few parameters were evaluated in relation to disease specific survival; no association was found.

Fowles JS, Denton CL, Gustafson DL: Comparative analysis of MAPK and PI3k/AKT pathway activation and inhibition in human and canine melanoma. Vet Comp Oncol. 2015 Sep;13(3):288-304 doi: 10.1111/vco.12044.

Objectives:

To compare the activation of the MAPK and PI3K/AKT pathways in both human and canine melanoma by Affymetrix-based gene expression, mutational analysis, and measures of constitutive activation as well as assessing effects of specific inhibitors to these pathways on melanoma cells lines across species.

Study Design:

Investigative and retrospective

SUBGROUP CONCLUSIONS:

This is not a prognostic study. The purpose of the present work was to analyze, by a comparative study, the activation of specific pathways known to be activated in human melanomas and that are targeted in current targeted therapies for human melanoma. Data reported are extremely interesting since, despite the fact that the mechanisms of activation of the pathways can be different between the two species, the activated pathways are the same and melanoma cells of both species are similarly sensitive to specific inhibitor molecules. Mutations were different between the two species which is in accordance with previous reports. Take away message is that the MAPK, PI3K/AKT pathway is likely active in canine melanoma and it may benefit from inhibition, but the driving mutations remain unidentified.

Abou Asa S: Immunohistochemical Expression of MCAM/CD146 in Canine Melanoma. J Comp Pathol. 2017 Jul;157(1):27-33. doi: 10.1016/j.jcpa.2017.05.001. Epub 2017 Jun 7.

Objectives:

To investigate the immunohistochemical expression of MCAM/CD146 in 51 canine melanomas, including oral, cutaneous and ocular tumours. MCAM/CD146 was identified originally as a

marker for human melanoma because it was overexpressed in metastatic lesions and advanced primary tumours, but was not expressed by benign lesions. The authors wanted to evaluate whether or not this marker is expressed in canine melanomas so that it could perhaps be used as a target for therapy.

Study Design:

Retrospective study using archived canine melanoma biopsy specimens.

SUBGROUP CONCLUSIONS:

This is not a prognostic study. This marker may hold promise for future targeted therapy but much additional research is needed before then.