VCS/ACVP Oncology-Pathology Working Group
Summary and Subgroup Recommendation for
Consensus on Grading Canine Soft Tissue Sarcomas

Species/Tumor: **CANINE SOFT TISSUE SARCOMAS SUBGROUP**

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Overall Summary / Recommendations:
Based on review of the literature, the ***Canine Soft Tissue Sarcoma Subgroup*** has concluded and recommends the following regarding _grading_ specifications for ***canine soft tissue sarcoma.*** As a consequence of our discussions, we have also proposed starting points for consideration for clinical follow up, diagnosis, margins, and new terminology (i.e. soft tissue mesenchymal tumor) as appendices to our primary statement paper on determination of a grading scheme for soft tissue sarcomas.

The selected literature is reviewed in the appropriate sections of the discussion.

Soft Tissue Sarcoma Subgroup
Consensus on Grading Canine Soft Tissue Sarcomas

Introduction
The soft tissue sarcoma committee of the Veterinary Cancer Society (VCS)/American
College of Veterinary Pathology (ACVP) Oncology Pathology Working Group (OPWG)
evaluated the usefulness of current grading systems for canine soft tissue sarcomas. After
review of the literature failed to reveal a robust and validated grading scheme, the
committee elected to describe not only where our current grading schemes fail, but also
how one might determine how to best grade canine soft tissue sarcomas. Not
unexpectedly, these conversations led to additional issues such as tumor nomenclature,
tumor diagnosis, margin evaluation and clinical follow-up. Short summaries of these
topics are included as appendices to the primary document. While these appendices were
reviewed by the committee, they are our current opinions, and do not represent a
complete review of the literature by this committee on these topics.

Shortcomings of Existing Grading Schemes for Canine Soft Tissue Sarcomas (STS)

Our review of published papers proposing grading schemes for canine soft tissue
sarcomas did not reveal any schemes which had been evaluated in more than one cohort
of samples, nor did the most commonly cited scheme, that proposed by Kuntz et al.,
evaluate any tumor histopathologic characteristics beyond those found prognostic in
human sarcomas.(*1-3*) The studies which reported using the grading scheme evaluated
by Kuntz et al. all used slightly different variations. This lack of standardization
effectively compromises comparisons across studies. In addition, the only retrospective
study which specifically evaluated the ability of tumor grade to predict outcome in
patients receiving only local therapy found no difference in the rate of metastasis between
low and high grade tumors.(*9*) Consideration should be given to using only one of the
current grading schemes (e.g. the scheme in the Kuntz paper or, even more simply, a
well-defined mitotic count and necrosis) in the interim until a more robust grading
scheme is developed.

The original veterinary sarcoma grading scheme was proposed by Bostock and
colleagues and relied upon "mitotic index" (now understood to be mitotic count) to
determine prognosis. Clinical follow-up, an essential component when developing a
prognostic grading scheme, consisted of monthly questionnaires to the owners and
requested necropsies at the time of death. While mitotic index was highly significant in
predicting local recurrence, metastasis and survival, it is unclear how many of the 187
dogs in the study had owners that participated in the monthly surveys or how many had
full or partial necropsies.

The Kuntz grading scheme proposed the use of three tumor characteristics: percent
necrosis, degree of differentiation and mitotic index. The grading scheme proposed by
Kuntz appears to be based upon one proposed in papers by Coindre and Trojani. These

papers are the foundation for the human sarcoma grading scheme.(*2, 3*) However, the materials and methods section of the Kuntz paper did not provide methods of how percent necrosis or details of how mitotic index (MI) were evaluated. We now know that MI was not performed, and in fact it was a mitotic count. Regardless, this grading scheme has been widely utilized and is a starting point for future studies, but histopathologic parameters need to be described in such detail that others can reproduce those methods and additional parameters, such as the seven proposed by Tojani et al., considered. Using the parameter of percent necrosis as an example, the original studies and current methodology used by MD pathologists includes the combination of gross evaluation of the tumor sample in addition to histologic examination in order to estimate necrosis. All veterinary studies we reviewed appeared to use only microscopic sections with no description of a gross examination, making it possible that the areas examined microscopically were not representative of necrosis. In addition, the grading scheme in the Kuntz study utilizes no necrosis and the cutoff of more or less than 50% necrosis for grading purposes (based upon the human parameters), but also states that a cutoff of 10% necrosis was statistically prognostic (dogs with >10% tumor necrosis were 2.7 times more likely to die of tumor related causes). We have seen no other studies that reference 10% necrosis as a prognostic parameter. Further studies need to detail a methodology that will accurately assess percent necrosis in the entire tumor in a way that allows others to reproduce those methods. Mitotic index in the Kuntz, McSporran and Bostock papers is defined as number of mitoses/10 HPF(*1, 4, 5*), but different ranges of MI were used in each study-- and this is incorrect usage of the term MI.(*6*) MI is an index and is determined by counting cells in mitosis (mitotic figures) divided by cells not in mitosis. This is tedious but correlates well with other parameters (Ki67, PCNA) that determine cell proliferation. Importantly, the area in which mitotic figures were enumerated was not defined in the above canine studies. Defining the area as *per 10 HPF* is not a standard unit of measurement as the area in 10 HPF can vary by 200%+ between microscopes/pathologists. The two most commonly cited human sarcoma studies define the area in which mitotic figures are counted and correctly report this as mitotic count (MC).(*2, 3*)   There is one veterinary study that defines and uses mitotic count correctly but appears to be seldom cited.(*7*) Over the years it appears that the term MI was incorrectly exchanged for MC in the veterinary literature and this usage has been perpetuated. In a recent letter to the editor in *Veterinary Pathology* (co-authored by one of the subgroup members) it has been proposed that the area in which mitotic figures are counted should be defined as $2.37mm^2$.(6) This area is determined easily in scanned images or using a microscope: 10 contiguous fields at 400X magnification using oculars with 22mm field diameter equals $2.37mm^2$. (6) If a microscope has oculars with a different FN then simple calculations can be applied to equate the area to $2.37mm^2$. The impact of using different field areas to count mitotic figures ranges from minimal to large, example: twice as many mitotic figures could be seen in 10 HPF (40X objective) with an ocular of FN 26.5mm as compared to 10 HPF (40X objective) with an ocular of 18mm. Modern microscopes have oculars with FN 20 or 22mm. The method by which the area is selected and the criterion of what constitutes a mitotic figure have been defined. (2, 6) When the field area is defined, how the area is selected and the definition of mitotic figure(s) are standardized and those criterion are followed we will be able to compare results between labs that used microscopes or scanned images to evaluate

tumors. Bostock stated they used a random field (free of necrosis) in which to start counting mitotic figures, McSporran chose the most cellular area, Kuntz, Coindre and Trojani did not state how the initial field was selected; but in a subsequent paper Coindre stated that the area counted should start in a region with the most mitotic activity. Bostock, Kuntz and McSporran reported MI but determined MC and did not define the area enumerated. This highlights the necessity to standardize methods.

In the Kuntz study, only mitotic count (reported as MI) was prognostic for the development of distant metastasis in the multivariate analysis. With respect to survival, only mitotic count and percent necrosis were prognostic in the multivariate analysis. Prognostic factors in the univariate analyses included mitotic count, degree of differentiation, tumor size and histopathologic grade (based on Bostock), however half of these were not significant on multivariate analysis. The evaluation of histopathologic characteristics takes time and the outcome can alter treatment. In addition, many characteristics are subjective, to a lesser or greater degree, leading to greater difficulty in their consistent application. Ideally, a grading scheme would include only those characteristics which are necessary and sufficient to differentiate sarcomas based upon clinical outcome. A grading scheme should also result in consistency between pathologists, and thus should be described in sufficient detail to allow others to reproduce the results. The evaluation of a standardized area, an appropriate region (e.g. not necrotic, highly cellular, at the periphery) of the tumor are critical, as is how percent necrosis is determined and how degree of differentiation is assessed, and are aspects of diagnosis and grading that must be clearly defined. All of these concerns arose from the committee's review of the sarcoma literature.

The generation of a grading scheme is additionally dependent upon accurate and complete clinical follow-up of all patients as well as clearly defined, study-related, clinical end-points (eg. disease free interval (DFI), overall survival (OS), etc). Thus, these parameters also need to be fully described in all studies (see Appendix 1). This information is lacking in current veterinary studies.

Recommendations for Developing a Grading Scheme for Canine Soft Tissue Sarcomas

The original paper describing the development of the grading scheme used for human soft tissue sarcomas might serve as an example of how a canine grading scheme can be established. Trojani and colleagues evaluated seven characteristics: tumor differentiation, tumor cellularity, atypical nuclei, malignant giant cells, MC, tumor necrosis and vascular emboli.(3) These seven characteristics were chosen based on the proposition in the literature that they were prognostic for outcome in patients with sarcomas. With the exception of MC and degree of differentiation, all characteristics were bimodal. Degree of differentiation was the most subjective parameter assessed. We would recommend a similar approach to develop a grading scheme for canine sarcomas: defining any parameters that may be predictive; analyzing the data statistically and then designing a final grading system which includes only characteristics necessary and sufficient to differentiate clinical outcome (practical final grading system). This newly

proposed grading scheme must then be prospectively validated in a second set of patients with sarcomas. In addition to the seven characteristics initially evaluated in the human sarcomas, other characteristics which could be considered for evaluation include gross measurements, completeness of excision, microvascular density, proliferation markers, nuclear pleomorphism, nucleolar prominence, multinucleate cells, cellularity, vascular invasion, or other nuclear or cytological characteristics (such as degree of cellular atypia).

Immunohistochemistry (IHC) is used in human sarcoma pathology to assist in diagnosis and subtyping, as well as to determine prognostic factors. In order to minimize the cost associated with assigning tumor grade for canine STS, we would propose that IHC be evaluated separately from tumor grade for canine STS, although clearly IHC may be necessary for diagnosis. Ideally, the same panel of antibodies should be applied to every case to avoid selection bias.

Once the pathological characteristics are evaluated, the individual characteristics should be examined for prognostic significance for time to first event, disease free interval, local recurrence, metastasis and death. Those characteristics which best predict disease and patient outcome will be determined by statistical analysis. Analytics such as a random forest analysis can be used to determine the strongest predictors of tumor behavior, and the minimum number of prognostic variables necessary to divide tumors into prognostic groups. Of critical importance is that the development of a grading scheme on a given set of tumors must be validated in a separate group of tumors. Only with validation can a grading scheme be used with confidence.

Inherent in this approach are two assumptions: First, all tumors should be treated with surgery alone. While this may prevent the inclusion of a significant number of high grade sarcomas due to the additional chemotherapy many receive, the inclusion of patients who receive chemotherapy makes evaluation of grade separate from treatment very difficult. Second, the margins of all resected tumors must be similarly assessed, given the high index of suspicion that entirety of excision is prognostic for local recurrence. The current assessment method, measuring the tumor free margin on H&E sections should be evaluated. A simpler approach to margin evaluation has been proposed, given the inaccuracies present in the absolute measurement approach due to tissue shrinkage post excision and lack of standardized trimming and measuring techniques—this discussion is presented in Appendix 3.(*8*)

There are likely other factors besides histologic grade which will prove prognostic for outcome in canine STS. Examples of such factors may be genetic signatures, size, chromosomal rearrangements, miRNA signatures, epigenetic changes, host factors and perhaps even cytologic appearance. Any study evaluating tumor grade should also strongly consider obtaining tissue for banking (beyond formalin-fixed paraffin-embedded tissue, including frozen and RNAlater preserved tissues, for example) such that future studies can look at the same cohort for additional potential prognostic factors.

Final Conclusion and Recommendations for Grading Canine Sarcomas
1. Current literature lacks appropriate studies to provide an accurate, reliable, and repeatable grading scheme.
2. Methodologies for establishing a scientifically sound grading scheme have been proposed.
3. Based on the shortcomings of the current literature, the subgroup cannot recommend one study over another for grading. We do, however, 1) recognize the need for pathologists and oncologists to treat dogs with sarcoma with all the information currently at our disposal and 2) accept the general principle that high-grade neoplasms are more aggressive than low-grade neoplasms. Until a more scientifically sound grading scheme is established, the subgroup proposes the histopathologic reporting of canine soft tissue sarcoma provided below (Appendix 5) and strongly emphasizes that clinicians and pathologists realize the limitations and inadequacies on which current grading schemes were founded and work together to correlate outcome assessments with gross and histologic parameters moving forward.

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*Papers reviewed by the STS Subgroup

All papers reviewed by STS Subgroup of the OPWG

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Introduction to Appendices

While the primary goal of the subgroup was to establish consensus on the methodology we, the veterinary profession, should adopt for grading, it was determined that none of the currently proposed and published grading methods or schemes could be endorsed due to deficiencies in the means by which they were originally developed. As such, the group, rather than propose adoption of an existing grading method, has redirected its focus to the proposal of future directions for developing an accurate, validated, and robust grading scheme, which will reliably prognosticate outcome. Pursuit of this endeavor lead us to and required discussion in additional areas including: 1) the nomenclature of canine STS, 2) the critical nature and appropriateness of clinical data and follow-up, 3) the necessity of consistency of diagnosis and subtyping, and 4) the significance of methodology of margin evaluation--as each relates to study design in the establishment of a reliable grading system. The primary consensus on grading was established based on thorough and critical review of the literature (references listed in the primary document) by all subgroup members **while appendices herein cite references, but are more of an opinion / starting point arising out of subgroup member discussions rather than extensive and critical review of the literature in these specific areas. These topics could also easily serve as sarcoma subgroup topics moving forward.**

Appendix 1: The Nomenclature of Canine Soft Tissue Sarcomas:

Changing a name needs justification and the authors wish first to explain why the name "soft tissue mesenchymal tumors" (STMT) may be preferable to soft tissue sarcomas. In the end, most canine tumors in this category are not malignant. Sarcoma implies the tumor is malignant and is expected to do one or more of the following: metastasize to a regional node, metastasize to lungs, metastasize to distant sites, recur and/or shorten the life of a patient. In fact, the majority of canine STS/STMT do not behave this way. Grading helps determine if a canine mesenchymal tumor will behave like a sarcoma (malignant) or an –oma (benign/less metastatic). The term and initials, STS, were adopted from human soft tissue sarcomas, the most common of which, by a wide margin, is liposarcoma, and it is malignant in its behavior. However, the majority of soft tissue mesenchymal tumors (STS/STMT) in dogs are not malignant.

Appendix 2: Clinical Data

Subgroup proposal for the collection of clinical data for future prognostic studies on canine STS

The accurate collection of clinical data is crucial for developing a canine STS grading scheme and allowing identification of prognostically relevant parameters. Clinical follow-up is the outcome assessment for determining which parameters are predictive of tumor behavior thus justifying inclusion into the grading scheme. Without

accurate, standardized outcome assessment it does not matter what the histopathological parameters are or how they were assessed. In the papers reviewed, there is considerable variation in the collection and reporting of clinical follow-up data, with the vast majority of data being retrospective in nature, thus preventing a meaningful comparison between publications. Clinical data must be collected with the same rigor applied to pathological data and standardization is requisite. Studies should document standard clinical parameters such as the presence, absence, and/or development of regional and distant metastasis as well as local recurrence in a standardized manner and at fixed time intervals. Information on all treatments the patient received should also be documented, although the development of the grading scheme ideally should be restricted to patients treated with surgery only. Data on the natural history of canine STS was found to be lacking in the veterinary literature (i.e. survival and metastatic rates for untreated canine STS are largely unknown). This lack of information, coupled with the subjective assessments associated with the timing of euthanasia in the veterinary patient population, renders interpretation of survival times problematic. Therefore, the inclusion of additional endpoints such as time to first event and disease-free interval must be reported, as these may represent more objective measures of treatment effects. General guidelines related to the collection and reporting of clinical data have already been published.(*9*) Specific application of these principles with further explanation as applicable to canine STS is provided below to encourage uniform use of terminology, transparency in data reporting, and comparisons among future studies. If survival times are reported, then the manuscript's methods section needs to clearly define if the patient was euthanized and how it was determined that the tumor contributed to the cause of death (e.g. owner could no longer administer nursing care, dog developed a new tumor, etc.). This information should be published in a table as supplementary data.

Studies should clearly define the treatment groups evaluated in the study. Differences between treatment groups should be minimized such that any observed outcome differences can be attributed to the treatments evaluated. Study design, with specific attention to number of groups and sample size, should be carefully considered to avoid type II statistical errors.(*10*) Manuscripts must provide detailed descriptions of the treatments used. Furthermore, patient tumor descriptions should be adequately detailed with appropriate staging information provided. In addition to complete staging information (standardized RECIST VCOG v1.0 measurements, locoregional lymph node status, and presence of metastasis), tumor location should be included as distally located STS may display a unique biologic behavior pattern.(*11*)

Local recurrence is defined as the presence of the same tumor within the region of the previous surgical site confirmed via histopathology.(12) There are multiple reasons that histopathology is required to confirm recurrence. Histology is required to exclude benign causes of a mass in the region of the surgical scar (e.g. reactive fibroplasia, gossypiboma) or unrelated *de novo* tumors (e.g. mast cell tumor). Additionally, cytological evaluation of fine needle aspirates cannot distinguish granulation tissue from STS. All recurrent masses should be measured by the clinician, surgeon, histotechnologist handling the gross specimen and trimming in the tissue, and pathologist. Local recurrence data should be stratified as either "confirmed" (i.e. histologically confirmed) or "presumed." Presumed local recurrences should be further described by clearly stating which diagnostic modalities were used in the study: fine

needle aspirate cytology, imaging (e.g. ultrasound, contrast-enhanced computed tomography, etc.) or palpation alone. Standard RECIST VCOG v1.0 criteria should be used in reporting recurrences. A local recurrence should be counted as a single event regardless of the number of tumor nodules that may have appeared at the original surgical site. If a STS arises at a different soft tissue site it should be categorized as a "de novo STS at a different site" and not a metastasis.

Metastasis should be evaluated both within the locoregional lymph nodes as well as the pulmonary parenchyma. Metastasis should be classified as "regional" (defined as locoregional lymph node involvement) or "distant." As with local recurrences, metastatic lesions should be stratified as either "confirmed" (i.e. histologically or cytologically confirmed, with histology preferred), "presumed," or "suspected." Presumed metastatic lesions should be further described by clearly stating which diagnostic modalities were used in the study: fine needle aspirate cytology, imaging (e.g. radiology, ultrasound, contrast-enhanced computed tomography, etc.) or palpation alone. When using radiography to evaluate for pulmonary metastasis, a minimum of 3 views (right lateral, left lateral, and ventro-dorsal or dorso-ventral) should be used. Computed tomography provides superior spatial resolution to radiography and therefore studies should clearly state which imaging methods were used for which body regions. Confirmation of suspected metastatic disease is accomplished by histopathology, which is the gold standard. If the metastatic lesion cannot be safely sampled, it should be categorized as "suspected." Reporting locoregional and pulmonary metastasis (or other organ metastasis) distinct from one another is important to facilitate identification of tumor biologic behavior patterns. Well-established methods do not currently exist for sentinel lymph node mapping with canine STS. As such, sampling of locoregional lymph nodes remains the standard method for testing for metastasis; this is expected to evolve with the development of new techniques in this field. Ideally, histology would be performed on all suspicious lymph nodes. If cytology of a lymph node is suspicious for metastatic STS, the node should be biopsied and submitted for histopathologic evaluation. The frequency of staging and follow-up should be standardized and performed on a fixed schedule to reduce bias (see below).

The recommended minimum follow-up time for studies involving canine STS soft tissue sarcomas is 24 months, with longer follow-ups preferred. Patients should be evaluated on a regular basis (e.g. every 3 months) to allow accurate determination of time to event following treatment. Shorter follow-up times are likely to report artificially low rates of recurrence and/or metastasis. This is due to the relatively long disease-free intervals that may occur before disease progression. (*1, 12*) Follow-up examinations should be performed in a standardized and complete manner, including: a complete physical examination with careful palpation of the treated site, locoregional lymph nodes, and imaging of the thoracic cavity via 3-view radiographs or computed tomography. Study participants with presumed metastatic and/or locally recurrent lesions may in fact have lesions unrelated to the primary tumor. Dogs with cancer have been documented to develop more than one tumor.(*13*) Therefore, results from studies with large proportions of presumed lesions should be interpreted with caution. It should be noted that increasing the post-mortem examination rate in studies is crucial to generating valid results by maximizing the number of dogs that are histologically evaluated for metastasis and/or local recurrence. In particular, high necropsy rates (e.g. minimum of 20% of patients) in

future studies is essential to definitively resolve the variation seen in metastatic and local recurrence rates reported in the current literature, many of which lack histologic lesion confirmation and/or necropsy data.(*1, 5, 12, 14-17*) Presently there is not accurate data to state what percent of STS metastasize to organs, lungs and or lymph nodes. To assist in ensuring post-mortem exams and histological confirmation of metastasis and or local recurrence, it should be included as a component of study design and its utility and significance described on the patient consent form as well as conveyed directly to owners choosing to enroll in a said study.

Studies should ensure that censoring of patient data is executed appropriately to maximize the value from survival curve analysis. Detailed recommendations can be found in the previously published guidelines on the collection and reporting of clinical data.(*9*) Briefly, patients should be right censored if they have not experienced a relevant event by the end of the study (e.g. still alive, no recurrence, no metastasis), or have been lost to follow-up. Censoring due to "death from other causes" should be avoided due to our inability in most cases to firmly rule out any relationship between the tumor, its treatment and the cause of death; unless a post-mortem exam by a board-certified veterinary anatomic pathologist is performed. Finally, in addition to the outcome data already discussed, studies should include 1-, 2-, and 5-year survival rates and should also include dogs whose owners have elected no treatment, in order to better describe the natural course of disease.

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Appendix 3: The Diagnosis and Classification of Canine Soft Tissue Sarcomas

Proposal for the diagnosis and classification of non-visceral soft tissue sarcomas (soft tissue mesenchymal tumors)

The parameters evaluated and subtyping / classification of canine STS has been inconsistent at different institutions and with changes in preferred classification over time. Current statistical data is insufficient to determine if tumor subtype is prognostic. It is necessary that we classify tumors consistently and use the same parameters to allow future studies to define the prognostic significance of STS and specific tumor subtypes.

The OPWG STS Subgroup proposes histopathology reports for STSs include a pattern diagnosis based upon the most recent and best-defined classification scheme. At the time of this writing, the subgroup proposes the classification of soft tissue neoplasms outlined by Dennis et al., in the 2011 review on canine soft tissue sarcomas (Vet Pathol 48:75 2011, table 1).   Tumors that cannot be classified based on pattern and cell differentiation should be referred to as undifferentiated sarcomas.   Peripheral nerve sheath tumors arising from the brachial plexus, histiocytic sarcoma, leiomyosarcoma, rhabdomyosarcoma, synovial cell sarcoma, visceral sarcomas, malignant melanoma, oral fibrosarcoma, lymphangiosarcoma and hemangiosarcoma are typically excluded from this categorization as their behavior has been previously characterized and/or they often involve sites other than the skin or subcutis. However, in any study that strives to improve the diagnosis of STS, it may be warranted to include any or all of these sarcoma subtypes; it is important that the criteria by which different subtypes are identified be clearly defined in the materials and methods.

In research studies reporting outcome and prognosis of canine STS, we further propose a consistent panel of immunohistochemical markers to provide additional support of the diagnosis. It is preferable this panel be applied to all tumors to avoid bias. A summary of immunohistochemical staining patterns is provided in Table 1, Dennis et al, 2011 and should be updated and selected by the study pathologist(s) as additional markers become available. Immunohistochemical markers should always be interpreted in light of histomorphology and electron microscopic findings.   Immunohistochemical markers are recommended to help define poorly differentiated lesions that may fall under an excluded diagnosis.   Tumors that lack a diagnostic pattern and that do not express any of the panel of diagnostic antibodies evaluated are classified as undifferentiated sarcomas. The study pathologists should develop a standardized materials and methods in such detail that each component in the study design can be replicated. Study pathologists should report interpathologist variation of diagnoses and how IHC or other parameters used changed diagnoses.

Reference: Dennis MM et al.   Prognostic factors for cutaneous and subcutaneous soft tissue sarcomas in dogs.   Vet Pathol 48:73-84, 2011.*

Table 1. Distinctions Among Types of Soft Tissue Sarcoma in the Dog

Type	Tissue of Origin (Histogenesis)	Phenotype	Histologic Hallmark[27,29]	Typical Immunohisto-chemistry (%)[a]
Fibrosarcoma	Fibrous tissue	Fibroblast, fibrocyte	Interwoven bundles, herring-bone pattern, pronounced collagenous stroma	
Keloidal fibrosarcoma	Fibrous tissue	Fibroblast, fibrocyte	Hyaline collagenous stroma[41]	
Myxosarcoma	Fibrous tissue		Stellate- or spindle-shaped cells in mucinous stroma	
Liposarcoma	Fat	Lipoblast, lipocyte	Polygonal cells with distinctly vacuolated cytoplasm	
Perivascular wall tumors (glomus tumor, hemangio-pericytoma, myoperi-cytoma, angioleiomyoma/sarcoma, angiomyofibro-lastoma, angiofibroma)	Cells of perivascular wall	Pericyte, myopericyte, smooth myocyte	Vascular growth patterns, including staghorn, placen-toid, perivascular whorling, and bundles from tunica media[2]	Calponin +[2] Pan actin +[2] SMA + (50%)[2] S100 −[2] NSE −[2] GFAP −[2] Myoglobin −[2]
Peripheral nerve sheath tumor (schwannoma or neurofibrosarcoma)	Peripheral nerve	Schwann cell, neurofibroblast	Interwoven bundles, whorls around collagen bundles, Antoni A and B patterns[47]	NSE + (45–82%)[12] S100 + (50–100%)[12,44,47] Neurofilament + (82%)[47] NGFR + (47%)[12] Myoglobin + (64%)[12] GFAP + (0–35%)[12,44]
Pleomorphic sarcoma (malig-nant fibrous histiocytoma)	Fibrous tissue	Primitive mesenchymal cells (potentially fibro-blast or myofibroblast)	Mixture of fibroblastic cells and karyomegalic, cytome-galic, or multinucleate his-tiocytoid cells in storiform patterns, with variable inflammatory infiltrate	Lysozyme + (29–100%)[44,51] MHC II + (70%)[43] Desmin + (86%)[51] S-100 −[51]
Mesenchymoma	Any mesenchymal tissue	Multiple cell types	Multiple soft tissue mesench-ymal cell types and matrix components, including osteoid, chondroid, collagen.	
Leiomyosarcoma[b]	Smooth muscle	Leiomyoblast, leiomyocyte	"Cigar-shaped" nuclei, promi-nent cytoplasm	SMA + Desmin + (100%)[44] Calponin + GFAP −[44]
Rhabdomyosarcoma[b]	Skeletal muscle	Skeletal myoblast, skeletal myocyte	Cytoplasmic striation, "racket" and "strap" cells	Myoglobin + Desmin + NSE + (50%)[12] GFAP + (50%)[12] S-100 + (75%)[12]

[a] All are typically vimentin positive. The summarized typical immunohistochemical findings for each soft tissue sarcoma type should be interpreted with caution. These values are summarized from studies that do not have uniform methods for immunohistochemical differentiation of each tumor or methods for inclusion criteria for selection of cases or case definitions. %, percentage of tumors that are immunopositive. GFAP, glial fibrillary acidic protein; SMA, smooth muscle actin; NSE, neuron-specific enolase; NGFR, nerve growth factor receptor.

[b] Most prognostic studies do not include these mesenchymal neoplasms of soft tissue in the cutaneous and subcutaneous soft tissue sarcoma grouping, because of differences in location of occurrence or metastatic rate.[17]

Appendix 4: The Evaluation of Surgical Margins in Canine STS

There are no studies in the veterinary literature which define what amount of surgical or microscopic margin is sufficient for the surgical treatment of STS; however, "completeness" of excision has been reported to be important in the outcome of these patients.

While the completeness of excision should be evaluated for prognostic significance, this requires input from the surgeon who may define the margins in situ, the trimmer of the fixed specimen and the pathologist in histologic sections.   Moreover, while standardized methods of tissue trimming and sectioning have been proposed (Kamstock et al 2011), the subject of surgical margins in canine STS is beyond the current scope of this consensus statement.

References: D A Kamstock et al. Recommended guidelines for submission, trimming, margin evaluation, and reporting of tumor biopsy specimens in veterinary surgical pathology.   Vet Pathol. January 2011;48(1):19-31.

Appendix 5: Subgroup's Proposed Grading for Canine Soft Tissue Sarcomas

Until a more scientifically robust and sound grading scheme is established, the subgroup suggests the following for histopathologic reporting of canine soft tissue sarcomas

While the sarcoma grading subgroup has found that none of the currently published grading schema have adequate data for endorsement for use in veterinary oncology, we do recognize the need for pathologists and oncologists to treat dogs with sarcomas with all the information currently at our disposal. There are a number of pathologic descriptors that have been suggested to be prognostic, including mitotic count, percent necrosis, and sarcoma subtype, among others. Moving forward, these descriptors and others should be investigated and must be validated in a robust fashion before they are widely adopted and or endorsed.   In the interim, the subgroup accepts the general principle that high-grade neoplasms are more aggressive than low-grade neoplasms and therefore proposes the following descriptors be included in histopathologic reports as a part of the discussion between oncologists and pathologists as we attempt to care for patients in the most appropriate fashion. If pathologists choose to grade these tumors we recommend following the scheme outlined below. Ultimately, however, full validation of any proposed grading scheme is critical.

In the interim, we propose the following be reported on sarcomas subjected to histopathologic review:

1. Diagnosis

2. MC as # per10 HPF (2.37 mm2) digital image or microscopic image
3. Grade
4. Margin

Suggested system to assign grade is based on references 1-5.

Differentiation score:
Score 1: Well-defined diagnostic pattern for classification
(well-differentiated)
Score 2: Poorly defined diagnostic pattern, but sufficient for classification
(poorly-differentiated)
Score 3: Undifferentiated sarcoma, sarcomas of unknown type

Mitotic count
Score 1: 0–9 mitoses per 10 HPF[*]
Score 2: 10–19 mitoses per 10 HPF
Score 3: ≥20 mitoses per 10 HPF

Tumor necrosis – assessed in histologic section(s)
Score 0: No necrosis
Score 1: ≤ 50% tumor necrosis
Score 2: > 50% tumor necrosis

Histologic grade: sum the scores
Low Grade: Total score 2, 3
Intermediate Grade: Total score 4, 5
High Grade: Total score ≥6

[*]10 high-power fields (HPF: 40X objective and 10X ocular with FN 22) measures 2.37 mm^2 with a microscope. Select an area at the periphery with the most active cellular proliferation.