Supplemental Data to the Manuscript `Willmann et al`:

Proposed Diagnostic Criteria and Classification of Canine Mast Cell Neoplasms: A Consensus Proposal

Table S1

Bostock Histopathological Grading System for Canine Cutaneous MC Tumors (1973)(1)

	Tumor Grade		
	I high grade	II intermediate grade	III low grade
Cell morphology	Highly cellular, un-differentiated cytoplasmic boundaries, irregular size and shape of nuclei, frequent mitoses, sparse cytoplasmic granules.	Cells closely packed with indistinct cytoplasmic boundaries, nucleus-to-cytoplasmic ratio lower than anaplastic, frequent mitoses, more granules than anaplastic.	Clearly defined cytoplasmic boundaries with regular, spheric, or ovoid nuclei; mitoses rare or absent; cytoplasmic granules large, deep staining, and abundant.

Table S2

Patnaik Morphologic Grading Classification for Canine Cutaneous MC Tumors (1984)(2)

	Tumor Grade		
	I	II	III
Location	Dermis and interfollicular Spaces	Infiltrate lower dermal and subcutaneous tissue; some extend to skeletal muscles or surrounding tissues	Replace subcutaneous and deep tissues
Cell morphology	Round, monomorphic, ample distinct cytoplasm with medium-sized granules	Round to ovoid, moderately pleomorphic, with scattered spindle and giant cells; most cells distinct cytoplasm with fine granules, but some with indistinct cytoplasm and large/hyperchromic granules	Round, ovoid, spindle shaped, pleomorphic, medium sized; cytoplasm indistinct with granules that are fine or not obvious; many giant cells and scattered multi-nucleated cells
Nuclear morphology	Round, condensed chromatin	Round to indented with scattered chromatin and single nucleoli; some with double nuclei	Indented to round vesiculated, with 1 or more prominent nucleoli; common bi-nucleated cells
Architecture, Cellularity, stromal Reaction	Arranged in rows or small groups, separated by mature collagen fibers of the dermis	Moderately to highly cellular; arranged in groups with thin fibrovascular stroma (some-times thick and fibro-collagenous with areas of hyalinization). Sometimes, neoplastic mast cells can infiltrate in the lower dermis or even subcutaneous tissue; some tumors may even infiltrate into the skeletal muscles or surrounding tissues	Cellular, arranged in closely packed sheets; stroma fibrovascular or thick and fibrocollagenous with areas of hyalinization
Mitotic figures	None	Rare (0-2/HPF)	Common (3-6/HPF)
Edema and necrosis	Minimal	Areas of diffuse edema and necrosis	Edema, hemorrhage, and and necrosis common

HPF, high-power field

Table S3

Kiupel Two-Tier Grading Criteria for Canine Cutaneous MC Tumors (2011)(3)

	Tumor Grade	
Criterion*	low grade	high grade*
Mitotic figures	< 7 MF/10 HPF	≥ 7 MF/10 HPF
Cell Morphology	< 3 multinucleated cells /10 HPF	≥ 3 multinucleated cells /10 HPF
Nuclear Morphology	< 3 bizarre nuclei/10 HPF	≥ 3 bizarre nuclei/10 HPF
Karyomegaly	< 10% of neoplastic cells	≥ 10% of neoplastic cells

*Each of these criteria is sufficient to assign a high grade. Abbreviations: MF, mitotic figures; HPF, high-power fields

Table S4

WHO staging system for canine cutaneous mast cell neoplasms (4)

Clinical Staging	Criteria
Stage I	One tumor confined to dermis without regional lymph node involvement
Stage II	One tumor confined to dermis, with regional lymph node involvement
Stage III	Multiple dermal tumors or large infiltrating tumor with or without regional lymph node involvement
Stage IV	Any tumor with distant metastasis or recurrence with metastasis

(4) Owen et al., 1980

Table S5

Proposed amendment to the WHO staging system for canine cutaneous MCTs (5)

Clinical Staging	Criteria
Stage I	Single tumor, without regional lymph node involvement
Stage II	Multiple tumors (≥3), without regional lymph node involvement
Stage III	Single tumor, with regional lymph node involvement
Stage IV	Large and infiltrative tumors, without delineation, or multiple tumors (≥3), with regional lymph node involvement
Stage V	Any tumor with distant metastasis, including bone marrow invasion and the presence of mast cells in the peripheral blood

Compared to the original WHO staging system (Table S4), Stage II presenting with multiple cutaneous tumors, was included (5).

Table S6

Clinical differences of the subvariants of cutaneous MCT

	Cutaneous MCT (3,34,35)	Subcutaneous MCT (19,36)
Recurrence rate	10.5% (3)	8% (19) – 9% (36)
Metastatic disease	18.7 (34) – 22.9% (3)	4% (19) – 5.7% (36)
MCT-associated death	11.6% (3) – 29.2% (35)	5% (36) – 9% (19)

MCT, mast cell tumor. Kiupel et al., 2011 (3); Thompson et al., 2011 (19), Stefanello et al., 2015 (34); Sabattini et al., 2015 (35); Newman et al., 2007 (36).

Table S7

Prognostic Markers for Canine Mast Cell Neoplasms

	References
Histologic Grade	Patnaik (2), Kiupel (3), Thamm (8), Webster (9), Giantin (10), Sledge (11)
Clinical stage	Krick (12), Warland (13), Worley (14), Murphy (15), Hillman (16), Horta (5)
Sentinel Lymph Node Involvement	Worley (14), Grimes (17), Fournier (18), Lapsley (19), Ferrari (20)
Proliferation Marker	
Mitotic Count	Kiupel (3), Horta (5), Thompson (21), Romansik (22), Thompson (23), Bertram (24), Vascellari (25), Berlato (26)
Ki67	Horta (5), Webster (9), Thompson (21), Vascellari (25), Berlato (26), Scase (27), Abadie (28), Seguin (29), Smith (30)
AgNOR	Thamm (8), Webster (9), Scase (27), Webster (31)
IHC KIT pattern	Horta (5), Giantin (10), Thompson (19), Kiupel (32), Reguera (33)
KIT Mutation	Horta (5), Giantin (10), Sledge (11), Webster (34), Takeuchi (35), London (36)
Other Variables	
Response to TKI treatment	Horta (37)

Ki67, Ki67 nuclear protein; IHC KIT pattern, immunohistochemical KIT pattern; AgNOR, Argyrophilic Nucleolar organizer regions.

Table S8

Comparison of cytological and histopathological evaluation of lymph node MCT metastasis according to Krick (6) and Weishaar (7)

Interpretation	Cytologic Criteria*	Histopathologic Criteria**	Histopathologic Classification
non-metastatic	No MCs seen	None to rare (0-3), scattered, individualized (isolated) MCs in sinuses (subcapsular, paracortical or medullary) and/or parenchyma per x400 field (0-3 MCs per x400 field), or does not meet criteria for any other classification below	HN0
Reactive lymphoid Hyperplasia	Greater than 50% small lymphocytes with a mixed population of prolymphocytes, lymphoblasts, plasma cells, and/or few to moderate numbers of macrophages, neutrophils, and eosinophils, and/or rare individual MCs		
Possible metastasis/ pre-metastatic	On at least one slide, two to three incidences of MCs in aggregates of two to three cells	Greater than 3 individualized (isolated) MCs in sinuses (subcapsular, paracortical or medullary) and/or parenchyma in a minimum of 4 x400 fields (unless otherwise stated at least 4 x400 fields each, which contain more than 3 MCs)	HN1
Probable metastasis/ early metastasis	On at least one slide, greater than three foci of MCs in aggregates of two to three cells and/or two to five aggregates of more than three MCs	Aggregates (clusters) of MCs (≥3 associated cells) in sinuses (subcapsular, paracortical or medullary) and/or parenchymal, or sinusoidal sheets of MCs	HN2
Certain metastasis/ overt metastasis	On at least one slide, effacement of lymphoid tissue by MCs, and/ or the presence of aggregated, poorly differentiated MCs with pleomorphism, anisocytosis, anisokaryosis, and/or decreased or variable granulation, and/or greater than five aggregates of more than three MCs	Disruption or effacement of normal nodal architecture by discrete foci, nodules, sheets or overt masses composed of MCs	HN3

*Krick et al., 2009 (6); **Weishaar et al., 2014 (7); MCs, mast cells; HN 0-3, histological nodal (HN) status 0-3

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