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# Features and prognostic impact of distant metastases in 45 dogs with *de novo* stage IV cutaneous mast cell tumors: a prospective study

## Abbreviated title

Canine stage IV cutaneous mast cell tumors

## Keywords

Mast cell tumor, metastases, stage IV, dog, outcome

## Abstract

Distant metastases in dogs with cutaneous mast cell tumors (cMCT) are rare and incurable. The clinico-pathological features of 45 stage IV cMCT dogs were prospectively investigated in relation to outcome. Dogs were uniformly staged and followed-up, whereas treatment was not standardized. Median survival time (ST) was 110 days. Notably, progression-free survival and ST were independent of well-known prognostic factors, including anatomic site, histological grade, and mutational status. Conversely, tumor diameter >3 cm, more than 2 metastatic sites, bone marrow infiltration, and lack of tumor control at the primary site were confirmed to be negative prognostic factors by multivariate analysis. Currently, there is no satisfactory treatment for stage IV cMCT. Asymptomatic dogs with tumor diameter <3 cm and a low tumor burden, without bone marrow infiltration may be candidates for multimodal treatment. The achievement of local tumor control seems to predict a better outcome in dogs with stage IV cMCT.

## Introduction

In dogs, cutaneous mast cell tumors (cMCTs) are clinically heterogeneous. Cutaneous MCTs may have a low malignant potential or be extremely aggressive, showing local invasiveness and having a high risk of metastases .[1] The most important independent prognostic factors are histological grading (according to Patnaik and Kiupel grading systems) and clinical stage, as they predict the biological behavior and provide reliable therapeutic indication.[2]

Surgery provides successful long-term outcome in dogs with well-differentiated, non-metastatic cMCTs.[1] If clean margins cannot be achieved, postoperative radiation therapy is also extremely successful for local control.[1] The vast majority of the clinical concerns of oncologists are related to the treatment of metastases, including how to eradicate, shrink or palliate the complications of metastatic disease.

A significant improvement in the locoregional control of cMCT has been seen over the last decades thanks to the advent of new antitumoral strategies and improved understanding of the biology of the disease. However, this improvement does not seem to have significantly influenced the final survival rate in dogs presenting with *de novo* stage IV disease, defined as dogs diagnosed with distant metastatic disease at initial presentation.[2-4]

The clinical relevance of nodal metastasis has been intensively explored. It results in a poorer clinical outcome according to several studies.[5-7] Particularly, histological rather than cytological lymph node (LN) staging is of crucial importance for estimation of prognosis and therapy stratification, as it is one of the strongest prognostic parameters.[2,5] In node-positive cMCT, systemic chemotherapy and/or tyrosine kinase inhibitors (TKIs) is generally recommended. In contrast, the benefit in stage I cMCTs is

minimal, and the decision of whether to use medical therapy depends on additional risk factors. Based on the above, the regional LN should always be assessed to determine the accurate stage of disease.[8]

Conversely, the clinical importance of distant metastases in cMCT has been investigated only in a few studies.[3,4] Recently it has been documented that approximately 5% of dogs with cMCT are diagnosed with distant metastases at initial presentation,[2] yet their prognostic relevance has not been intensively explored, as their disease is considered incurable, leading to palliative treatments and/or early euthanasia. Indeed, current information on the prognostic value of distant metastases is largely dependent on retrospective series that have been collected during several years and at multiple institutions.[3,4,9] Staging procedures as well as molecular analysis underwent a substantial change in recent years, leading to the need of reconsidering the relevance of new findings for dogs with metastatic disease.

The aims of this prospective study were to clarify the features of distant metastases of WHO stage IV cMCTs and to identify the prognostic factors for these dogs.

## Material and methods

### Inclusion criteria

Members of xxx were asked to participate to this prospective, multi-institutional study. Dogs were eligible for recruitment if they had a previously untreated, histologically confirmed cMCT and if they underwent complete staging demonstrating stage IV disease.

Background information recorded for each dog included signalment, body weight, primary tumor description (anatomic location, largest diameter, grade according to the systems of Patnaik and Kiupel, *c-kit* mutational status),[10-12] date of initial observation of primary cMCT; clinical stage and substage; date of stage IV diagnosis,; site of metastasis; date of surgery or incisional biopsy; histological margins status (for surgically resected cMCTs); other adjuvant treatments; response of the primary tumor to treatment; response of the metastatic sites to treatment; date of death or last follow-up examination; cause of death; and occurrence of treatment-related toxicity.

Initial staging included history and physical examination, complete blood cell count with differential, serum biochemistry, coagulation profile, histological examination of the cutaneous nodule, histological or cytological examination of regional LN, thoracic radiographs (3 views) and abdominal ultrasound examination or total body computed tomography (TBCT), fine-needle aspirates of liver and spleen regardless of their sonographic appearance, and cytologic examination of bone marrow (BM) obtained from the iliac crest.

The regional LN was defined as the first LN in the expected lymphatic drainage, and was identified either by palpation or by means of ultrasound. Cytologically, LNs or viscera were considered metastatic, if mast cells appeared in clusters or sheets, in very large numbers or atypical on morphology, as previously documented.[3] Histologically, LNs were considered metastatic in the presence of aggregates of mast cells in sinuses (subcapsular, paracortical or medullary) or parenchyma. Giemsa stain was applied in the uncertain cases. Bone marrow was considered infiltrated if mast cells were more than 10% of all nucleated cell, or, if atypical, more than 5 % of all nucleated cell, as previously described.[9]

Written informed consent was obtained from all owners.

## Treatment and response

The type of treatment was at the investigator's personal discretion, and included no therapy, surgery, radiation therapy, chemotherapy, TKI or a combination of these. Depending on treatment, dogs were re-assessed as follows: on a weekly basis if vinblastine was administered, on a monthly basis if lomustine, TKI or no treatment was administered. Physical examination, fine-needle aspiration of any new lesion, and bloodwork were routine elements of each assessment. An abdominal ultrasound was repeated every 1-2 months. All responses were defined according to the RECIST criteria.[13] Response was confirmed at least 4 weeks (for complete remission, CR, or partial remission, PR) or 6 weeks (for stable disease, SD) after the first documentation. Local tumor control was defined as objective local tumor response in addition to freedom from local progression. Distant tumor control was defined as objective distant tumor response in addition to freedom from distant progression.

## Statistical analysis

Progression free interval (PFI) was calculated from the date of stage IV diagnosis to the date of loco-regional and/or distant tumor progression. Survival time (ST) was calculated from the date of stage IV diagnosis to the date of last visit or death. Dogs lost to follow-up or dead due to MCT-unrelated causes were right-censored at the last date of known status or at the date of death, respectively.

The following factors were investigated for prognostic significance: age, sex, weight, anatomic location of primary tumor (site associated with a worse prognosis [head and neck, inguinal/ perineal region, digits] versus site associated with a better prognosis [trunk, abdominal wall, limbs excluding digital tumors), primary tumor diameter, regional LN metastasis, number of metastatic sites, BM infiltration, substage, histopathological grade (Patnaik and Kiupel), *c-kit* mutational status, measurable

primary tumor, type of treatment (surgery vs radiation therapy vs medical treatment), type of medical treatment (chemotherapy vs TKI), treatment-related toxicity, local and distant tumor control.

The influence of these factors on PFI and ST was investigated with a univariate Cox regression analysis. Median PFI and ST were assessed by means of the Kaplan-Meier survival plots. Factors that on univariate analysis had a P value < 0.05 were further tested for independence in a multivariate Cox proportional hazard model.

Statistical analysis was performed with SPSS Statistics v.19 (IBM, Somers, NY, USA). Significance was set at $P < 0.05$.

## Results

### *Dogs and MCT Demographics*

Between 2011 and 2016, 45 dogs matched the inclusion criteria and were enrolled. There were 17 (37.8%) mixed breed dogs, 7 (15.6%) Labrador Retrievers, 4 (8.9%) Boxers, 3 (6.7%) French Bouledogue, 2 (4.4%) Yorkshire terrier, 2 (4.4%) Maltese, and one (2.2%) each of the following: Shih-Tzu, Beagle, American Staffordshire terrier, Dobermann, Pinscher, Argentine Mastiff, Bullmastiff, Pitbull terrier, Boston terrier, and Malinois. Twenty-three (51.1%) dogs were female (14 spayed), and 22 (48.9%) were male (6 castrated). Median age was 9 years (range, 2 to 14 years), and median weight was 25 kg (range, 2.5 to 47.5 kg).

The cutaneous masses had been evident for a median of 11 days (range, 1 to 90 days). In particular, 32 cMCT had been evident for < 1 month, and 13 cMCT had been evident for 1 to 3 months. Mast cell tumors were in various locations, including 11 (24.4.%) dogs with MCTs on head and neck, 10 (22.2%) dogs with tumors on proximal

limbs (above elbow/ knee), 6 (13.3%) dogs with MCTs on the thoracic wall, 3 (6.7%) dogs with digital tumors, 3 (6.7%) dogs with mammary MCTs, 3 (6.7%) dogs with tumors on the scrotum, 3 (6.7%) dogs with tumors on the prepuce, 2 (4.4%) dogs with MCTs on the abdominal wall, 2 (4.4%) dogs with axillary MCTs, 1 dog (2.2%) with MCT on the vulva and 1 dog (2.2%) with MCT on distal limb (distal to knee/ elbow).

Median tumor diameter was 3.2 cm (range, 0.3 to 20 cm).

Twenty-six (57.8%) dogs were asymptomatic (substage a), whereas 19 (42.2%) dogs showed clinical signs and symptoms at diagnosis of stage IV disease (substage b), including vomiting, diarrhea, localized and/or generalized pruritus, and regional edema.

All dogs underwent complete staging work-up, as previously described; 36 (80%) dogs underwent three-view thoracic radiographs and abdominal ultrasound, whereas 9 (20%) dogs had a TBCT performed.

All dogs had distant metastatic disease: 22 (48.9%) dogs had splenic and hepatic metastasis, 6 (13.3%) dogs had hepatic metastasis, 3 (6.7%) dogs had splenic metastasis, 3 (6.7%) dogs had metastases in the spleen, liver and non-regional LNs, 3 (6.7%) dogs had metastases in the spleen, liver and BM, 2 (4.4%) dogs had metastases in the spleen and BM, 2 (4.4%) had metastases in the spleen, liver, BM, and non-regional LNs, 1 (2.2%) had metastases in the spleen, liver, BM and peripheral blood, 1 (2.2%) had splenic, hepatic and pulmonary metastases, 1 (2.2%) had metastases in the spleen and non-regional LNs, and 1 (2.2%) had splenic, renal and BM metastases.

Forty-one (91.1%) dogs had also metastasis in the regional LN, while 4 (8.9%) dogs did not. In these 4 dogs, the cMCT was located on the thoracic wall (n=2), on the vulva (n=1), and close to a mammary gland (n=1). Lymph node metastases were confirmed

in 30 (73.1%) dogs by means of histopathology, whereas the remaining 11 (26.8%) dogs had only a cytologic diagnosis. Regarding the 4 dogs without LN metastasis, the diagnosis was by means of histopathology in 3 (75%) of them, and by means of cytology in 1 (25%) dog. Visceral metastases were confirmed in all cases by means of cytology.

The median time between the first appearance of cMCT and diagnosis of stage IV disease was 22 days (range, 1 to 96 days).

Histopathology was available for all primary cMCTs: 23 (51.1%) dogs had Patnaik's grade 2 cMCTs, 20 (44.4%) dogs had grade 3 MCTs, and 2 (4.4%) dogs had grade 1 cMCTs. Regarding the Kiupel's grading system, 29 (64.4%) tumors were classified as high grade cMCTs, and 16 (35.6%) as low grade cMCTs.

Tissue specimens of all dogs were suitable for *c-kit* genotyping.

Overall, 16 (35.6%) cMCT had missense mutations. An exon 11 mutation was detected in 10 cases (8 internal tandem duplications [ITDs] and 2 single nucleotide polymorphisms [SNPs]), 4 tumors had both exons 11 and 12 ITDs, one dog had concurrently an exon 11 deletion and an exon 8 insertion, whereas the remaining case had an exon 8 ITD. Eight (17.8%) silent single nucleotide polymorphisms (SNPs) were detected in exon 8. Twenty-one (46.7%) dogs had wild type (WT) genotype (exons 8, 9, 11, and 12).

***Treatment and clinical follow-up***

Surgery was the primary treatment for 31 (68.9%) dogs; in 18 of them the MCT recurred shortly (within 30 days) postoperatively and those dogs had consequently macroscopic disease when first referred. All 18 cMCT that recurred had been removed

with dirty margins. Twenty-eight of the 31 dogs also received systemic treatment postoperatively, while 2 of 31 dogs also received curative-intent radiation therapy. Curative-intent radiation therapy ranged from 14 to 16 fractions for a total dose of 45 to 48 Gy.

Eleven (24.4%) of 45 dogs only received medical treatment as primary therapy.

Three (6.7%) of 45 dogs received a combination of palliative radiation therapy and systemic treatment as primary therapy. Palliative radiation therapy consisted of 5 fractions of 6 to 8 Gy each.

Overall, 42 (93.3%) dogs received systemic therapy, consisting of TKIs (n=22), dose-intense chemotherapy (n=7), or a combination of these (n=13).

Thirteen (30.9%) out of the 42 dogs receiving medical treatment experienced treatment-related toxicity, consisting of grade 1 lethargy (n=1), grade 1 (n=2), 2 (n=2) and 3 (n=2) gastro-intestinal side effects, grade 1 (n=1), 2 (n=1) and 3 (n=2) hematologic toxicity, and grade 2 hepatotoxicity (n=2).

When evaluating the primary MCT, 32 (71.1%) dogs had measurable disease and were therefore assessable for antitumor response. Three (9.4%) dogs achieved CR, 12 (37.5%) dogs experienced PR, in 4 (12.5%) dogs the primary disease was stable, whereas in 13 (40.6%) dogs was progressive, for an overall response rate in the macroscopic setting of 46.9%.

When considering metastatic disease in this group of dogs (including nodal and visceral), 3 (9.4%) dogs achieved CR at their metastatic sites, 2 (6.3%) dogs achieved PR, 6 (18.8%) dogs obtained SD, and 21 (65.6%) dogs progressed. Complete response was documented by imaging and confirmative cytology.

None of the 13 (28.9%) dogs with surgically removed cMCT without postoperative recurrence progressed at the primary site during the study period.

When considering metastatic disease in this group of dogs (including nodal and visceral), 5 (38.5%) dogs obtained CR, 3 (23.1%) dogs obtained PR, 4 (30.8%) dogs were stable, and 1 (7.7%) dog progressed. Overall, median PFI was 45 days (95% CI, 9.3-80.7 days).

The median follow-up time was 555 days (range, 142 to 1324 days). Forty-one (91.1%) dogs died or were euthanized within the follow-up period; among them, 40 died because of MCT-related disease and 1 because of a brain tumor after 380 days. Four (8.9%) dogs were alive at the end of the study. Overall, median ST was 110 days (95% CI, 67.9 to 152.1 days).

***Analysis of prognostic factors***

In univariate analysis, factors significantly associated with PFI were: tumor diameter >3 cm, more than 2 metastatic sites, regional LN metastasis, substage b, and measurable primary tumor (Table 1).

Factors significantly associated with ST were: tumor diameter >3 cm, more than 2 metastatic sites, regional LN metastases, BM infiltration, substage b, and lack of local and distant tumor control (Table 2).

In multivariate analysis, tumor diameter >3 cm, more than 2 metastatic sites and measurable primary tumor at diagnosis of stage IV disease were still significantly associated with PFI, whereas the factors associated with ST were BM infiltration and lack of local tumor control (Tables 3 and 4).

Age, sex, weight, anatomic location of the primary tumor, histopathological grade, mutational status, type of treatment, and onset of treatment-related toxicity were not significantly associated with either PFI or ST.

## Discussion

In one study, approximately 5% of dogs with cMCT had distant metastasis at initial diagnosis, with liver, spleen, BM and distant LNs being the major sites of metastatic involvement.[2]

Distant metastases are for most solid tumors decisive life-threatening events. Up to date, based on the recent literature, stage IV cMCT is perceived to be a very aggressive and ominous disease carrying a poor prognosis, with reported survival times ranging from 34 to 100 days among a total of 31 dogs examined in 3 studies.[3,4,9] Unfortunately, the studies published so far have only marginally improved the understanding of the outcome of dogs with stage IV disease, as no systematic body of knowledge on the clinical features, diagnosis, or treatment of such cases is available. Importantly, there are no guidelines on how to manage dogs presenting with stage IV cMCT, and decisions are often left to provider and owner preferences.

To our knowledge, this is the largest case series of dogs with de novo stage IV cMCT enrolled prospectively, uniformly staged and followed-up.

In this study, dog characteristics were similar to previous publications with median age of diagnosis of 9 years and no sex predilection.[8] In agreement with the literature, Labrador retrievers were over-represented.[14] While Boxers on the whole have been described to carry a better prognosis,[1,8,10] in the current study 8.9% of dogs were

Boxers, suggesting that the biologic behavior cannot be entirely anticipated by the signalment.

The same holds true for anatomic site of primary tumor development. While 24 of 45 dogs (53.3%) had MCTs that developed in sites described to behave in a more malignant fashion,[8] 21 (46.7%) did not.

Of utmost importance is the variability of grading that was documented in this series of dogs. Twenty-three (51.1%) dogs had Patnaik grade 1 and 2 cMCTs, and 16 (35.6%) had Kiupel low-grade cMCTs, thereby limiting the utility of histologic evaluation as the sole predictor of outcome in dogs with stage IV disease.[2] In addition, the statistical evaluation confirmed the non-prognostic role of histological grade in the presence of verified metastatic disease, leading to hypothesize that the histopathological evaluation might not be so essential for stage IV disease.

It has been reported that cMCTs harboring *c-kit* mutations, particularly some ITDs, have a poorer prognosis compared to those with WT *c-kit* genes.[15-17] Mutational status was documented in all dogs, and 21 (46.7%) dogs had WT genotyping (exons 8, 9, 11, and 12).

These data support that multiple variables need to be taken into consideration when predicting the biological behavior of cMCTs. If all dogs with MCTs are not staged there is a risk of missing dogs with stage IV disease.

Our clinical data confirm the poor outcome of stage IV disease, with a median ST of 110 days. Nevertheless, based on our results, the diagnosis of distant metastatic disease is not always necessarily a death sentence, as selected dogs may enjoy prolonged survival, clearly suggesting that additional factors in concert need to be taken into account to define prognosis.

Indeed, the current study identified some prognostic indicators in dogs with stage IV cMCT. While the PFI and ST for this group of dogs were largely independent of well-

known prognostic factors, such as anatomic site, histological grade, and mutational status,[10,11,15,19,20] some reported negative prognostic factors were confirmed.

That presence of systemic symptoms is associated with outcome has been already verified.[8,21] In our study, substage was an indicator of PFI and ST by univariate analysis; however, this relationship was not confirmed by multivariate analysis.

In agreement with previous studies, dogs with cMCTs larger than 3 cm had a significantly shorter PFI and ST.[2,21,22] The relationship with PFI was confirmed as independent factor by multivariate analysis. Accordingly, the presence of measurable primary MCT at diagnosis of stage IV disease was associated with significantly shorter PFI by multivariate analysis. In 18 of these 31 dogs, the measurable tumor represented recurrent disease shortly after a first surgery. As a whole, these results show that bulky disease may not be amenable to efficient local treatment, thereby worsening prognosis.

Metastatic burden also had a negative influence on PFI and ST according to univariate analysis, with more than 2 metastatic sites being associated with a poor outcome, but was not confirmed as independent factor for ST by multivariate analysis.

The concept of the regional LN has been already validated in dogs with cMCT.[5,23] In general, tumor cells at a specific anatomical site will first drain preferentially to the corresponding LN before reaching other LNs in the same regional basin and then spreading to distant sites, thereby following an orderly progression from local tumor growth at the primary site to the regional LN, followed by distant metastatic dissemination.[24] As a result, close examination of the regional LN is critical, as this provides valuable clinical information regarding the status of disease progression.[5-7]

Occasionally, cancer cells can spread to the systemic sites alone via the vascular system, thereby skipping the regional LN.[25]

In the current series, 4 of 45 (8.9%) dogs had no regional LN involvement despite the presence of distant metastases. Three of them underwent lymphadenectomy and histopathological evaluation, whereas one dog had cytological evaluation only. However, this dog was serially evaluated by means of LN cytology sampling during follow-up visits to confirm the absence of nodal metastasis (data not shown). Nodal metastasis was associated with ST, with dogs without LN metastasis having a longer PFI (940 versus 42 days, respectively) and living significantly longer (940 versus 109 days, respectively) than those with nodal disease. The biologic role of nodal metastases and their paradigm of orderly progression in cancer spread remains to be defined, but according to our results it may be possible that a small percentage of cMCT have different mechanisms of disease spread.

As already documented,[9] BM infiltration has important biologic implications, and was significantly associated with shorter ST by multivariate analysis. Disseminated tumor cells found in the BM may serve as reservoir of dormant cancer cells, representing the founder cells of overt metastases.[26] In general, disseminated cancer cells are considered to have a more aggressive phenotype, as they have developed the ability to home and survive in the BM, and evade the host immune recognition at the regional LN, being then able to colonize distant sites.[26] Of note, only 1 of the 9 dogs (11.1%) with BM infiltration had concurrent circulating neoplastic cells. This is in agreement with the human literature, showing that circulating neoplastic cells are numerically fewer than disseminated neoplastic cells, thereby requiring extremely sensitive analytical methods for their detection.[27,28] As a consequence, BM should be considered in dogs with nodal and/or visceral metastasis, as it indicates a higher

tumor burden and a worse prognosis (median ST 35 days vs 146 days, with and without BM infiltration, respectively).

Lack of tumor control at the primary and distant sites was significantly associated with ST; however only tumor control at the primary site retained significance by multivariate analysis. The variability in outcome is in part dependent on the type of treatment. The dogs receiving local treatment (surgery and/or radiation therapy) plus systemic treatment (chemotherapy or TKI or both) had a better outcome than those that did not. Similar results have been observed in a previous study,[14] suggesting that surgical resection of the primary cMCT followed by systemic therapy offers a significant survival advantage compared to dogs receiving chemotherapy without local control.

This study has some limitations. Although LN metastases were confirmed or ruled out by means of histopathology in the majority (73.3%) of cases, visceral metastases were confirmed by cytology in all cases. Nevertheless, the presence of several aggregates of mast cells, and their atypical morphology rendered the hypothesis of non-neoplastic mast cells unlikely in these cases.
Within our series, there was heterogeneity of treatment, as many owners elected not to pursue aggressive approaches to management due to the poor prognosis.

In conclusion, stage IV cMCTs are rare and associated with a poor outcome. Nevertheless, asymptomatic dogs with tumor diameter <3 cm and a low tumor burden, without BM infiltration may be candidates for local treatment plus systemic treatment. Stage IV dogs without LN metastasis may enjoy a surprisingly prolonged survival. The achievement of local tumor control seems to be the main predictor of

390 better outcome in dogs with stage IV cMCT.

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## References


1. London CA and Thamm DH. Mast cell tumours. In: *Withrow & MacEwen's Small Animal Oncology*. 5edn. SJ Withrow, DM Vail and Page RL Eds, Philadelphia, Saunders, 2013: 335-355.
2. Stefanello D, Buracco P, Sabattini S, Finotello R, Giudice C, Grieco V, *et al*. Comparison of 2- and 3-category histologic grading systems for predicting the presence of metastasis at the time of initial evaluation in dogs with cutaneous mast cell tumors: 386 cases (2009-2014). *Journal of the American Veterinary Medical Association* 2015; **246**: 765-769.
3. Stefanello D, Valenti P, Faverzani S, Bronzo V, Fiorbianco V, Pinto da Cunha N, *et al*. Ultrasound-guided cytology of spleen and liver: a prognostic tool in canine cutaneous mast cell tumor. *Journal of Veterinary Internal Medicine* 2009; **23**: 1051-1057.
4. Book AP, Fidel J, Wills T, Bryan J, Sellon R and Mattoon J. Correlation of ultrasound findings, liver and spleen cytology, and prognosis in the clinical staging of high metastatic risk canine mast cell tumors. *Veterinary Radiology & Ultrasound* 2011; **52**: 548-554.
5. Weishaar KM, Thamm DH, Worley DR and Kamstock DA. Correlation of nodal mast cells with clinical outcome in dogs with mast cell tumour and a proposed classification system for the evaluation of node metastasis. *Journal of Comparative Pathology* 2014; **151**: 329-338.
6. Krick EL, Billings AP, Shofer FS, Watanabe S and Sorenmo KU. Cytological lymph node evaluation in dogs with mast cell tumours: association with grade and survival. *Veterinary and Comparative Oncology* 2009; **7**:130-138.

7. Murphy S, Sparkes AH, Blunden AS, Brearley MJ and Smith KC. Effects of stage and number of tumours on prognosis of dogs with cutaneous mast cell tumours. *Veterinary Record* 2006; **158**: 287-291.
8. Blackwood L, Murphy S, Buracco P, De Vos JP, De Fornel-Thibaud P, Hirschberger J *et al*. European consensus document on mast cell tumours in dogs and cats. *Veterinary and Comparative Oncology* 2012; **10**: e1-e29.
9. Marconato L, Bettini G, Giacoboni C, Romanelli G, Cesari A, Zatelli A *et al*. Clinicopathological features and outcome for dogs with mast cell tumors and bone marrow involvement. *Journal of Veterinary Internal Medicine* 2008; **22**:1001-1007.
10. Patnaik AK, Ehler WJ and MacEwen EG. Canine cutaneous mast cell tumor: morphologic grading and survival time in 83 dogs. *Veterinary Pathology* 1984; **21**: 469-474.
11. Kiupel M, Webster JD, Bailey KL, Best S, DeLay J, Detrisac Cj *et al*. Proposal of a 2-tier histologic grading system for canine cutaneous mast cell tumors to more accurately predict biological behavior. *Veterinary Pathology* 2011; **48**: 147-155.
12. Marconato L, Zorzan E, Giantin M, Di Palma S, Cancedda S and Dacasto M. Concordance of c-kit mutational status in matched primary and metastatic cutaneous canine mast cell tumors at baseline. *Journal of Veterinary Internal Medicine* 2014; **28**: 547-553.
13. Nguyen SM, Thamm DH, Vail DM and London CA. Response evaluation criteria for solid tumours in dogs (v1.0): a Veterinary Cooperative Oncology Group (VCOG) consensus document. *Veterinary and Comparative Oncology* 2015; **13**: 176-183.
14. Miller RL, Van Lelyveld S, Warland J, Dobson JM and Foale RD. A retrospective review of treatment and response of high-risk mast cell tumours in dogs. *Veterinary and Comparative Oncology*. 2014; DOI: 10.1111/vco.12116. In press.

15. Downing S, Chien MB, Kass PH, Moore PE and London CA. Prevalence and importance of internal tandem duplications in exons 11 and 12 of c-kit in mast cell tumors of dogs. *American Journal of Veterinary Research* 2002; **63**:1718-1723.
16. Webster JD, Yuzbasiyan-Gurkan V, Kaneene JB, Miller R, Resau JH and Kiupel M. The role of c-KIT in tumorigenesis: evaluation in canine cutaneous mast cell tumors. *Neoplasia* 2006; **8**:104-111.
17. Zemke D, Yamini B and Yuzbasiyan-Gurkan V. Mutations in the iuxtamembrane domain of c-KIT are associated with higher grade mast cell tumors in dogs. *Veterinary Pathology* 2002; **39**: 529-535.
18. White CR, Hohenhaus AE, Kelsey J and Procter-Gray E. Cutaneous MCTs: associations with spay/neuter status, breed, body size, and phylogenetic cluster. *Journal of the American Animal Hospital Association* 2011; **47**: 210-216.
19. Dobson JM. Breed-predispositions to cancer in pedigree dogs. *International Scholarly Research Notice Veterinary Science* 2013; 941275.
20. Kiupel M, Webster JD, Miller RA and Kaneene JB. Impact of tumour depth, tumour location and multiple synchronous masses on the prognosis of canine cutaneous mast cell tumours. *Journal of Veterinary Medicine Series A* 2005; **52**: 280-286.
21. Mullins MN, Dernell WS, Withrow SJ, Ehrhart EJ, Thamm DH and Lana SE. Evaluation of prognostic factors associated with outcome in dogs with multiple cutaneous mast cell tumours treated with surgery with and without adjuvant treatment: 54 cases (1998–2004). *Journal of the American Veterinary Medical Association* 2006; **228**: 91–95.
22. Hahn KA, King GK and Carreras JK. Efficacy of radiation therapy for incompletely resected grade-III mast cell tumors in dogs: 31 cases (1987–1998). *Journal of the American Veterinary Medical Association* 2004; **224**: 79–82.

23. Worley DR. Incorporation of sentinel lymph node mapping in dogs with mast cell tumours: 20 consecutive procedures. *Veterinary and Comparative Oncology* 2014; **12**: 215-226.

24. Hellman S. Karnofsky Memorial Lecture. Natural history of small breast cancers. *Journal of Clinical Oncology* 1994; **12**: 2229-2234.

25. Fisher B. Laboratory and clinical research in breast cancer – a personal adventure: the David A. Karnofsky memorial lecture. *Cancer Research* 1980; **40**: 3863-3874.

26. Lin H, Balic M, Zheng S, Datar R and Cote RJ. Disseminated and circulating tumor cells: Role in effective cancer management. *Critical Reviews in Oncology/Hematology* 2011; **77**: 1-11.

27. Riethdorf, S, Wikman H, Pantel K. Review: Biological relevance of disseminated tumor cells in cancer patients. *International Journal of Cancer*; **123**: 1991-2006.

28. Pantel, K, Alix-Panabières C and Riethdorf S. Cancer micrometastases. *Nature Reviews Clinical Oncology* 2009; **6**: 339–351.

483 **Table 1** – Univariate Cox regression analysis of variables potentially associated with

484 increased risk of disease progression in 45 dogs with stage IV cutaneous mast cell tumors.

| **Variable** | **No. of dogs** | **Median PFI** (days) | **HR** | **95% CI** | ***P*** |
|---|---|---|---|---|---|
| Age | | | 1.33 | 0.71-2.51 | 0.373 |
| *> 9 years‡* | 21 | 36 | | | |
| *≤ 9 years* | 24 | 81 | | | |
| Sex | | | 1.21 | 0.64-2.29 | 0.551 |
| *male* | 22 | 29 | | | |
| *female* | 23 | 81 | | | |
| Neutered | | | 1.01 | 0.53-1.91 | 0.993 |
| *yes* | 20 | 42 | | | |
| *no* | 25 | 45 | | | |
| Weight | | | 1.37 | 0.72-2.60 | 0.339 |
| *≤ 25 kg‡* | 23 | 46 | | | |
| *> 25 kg* | 22 | 41 | | | |
| Anatomic site associated with a worse prognosis | | | 1.01 | 0.54-1.91 | 0.976 |
| *no* | 21 | 46 | | | |
| *yes* | 24 | 42 | | | |
| Tumor diameter at presentation | | | 2.79 | 1.38-5.64 | 0.004* |
| *> 3 cm* | 24 | 29 | | | |
| *≤ 3 cm* | 21 | 125 | | | |
| More than 2 metastatic sites | | | 3.03 | 1.30-7.03 | 0.010* |

| | | | | | |
|---|---|---|---|---|---|
| *yes* | 33 | 35 | | | |
| *no* | 12 | 84 | | | |
| Regional lymph node metastasis | | | 34.93 | 1.04-1167.96 | 0.047* |
| *yes* | 41 | 42 | | | |
| *no* | 4 | 940 | | | |
| Bone marrow infiltration | | | 1.70 | 0.73-3.98 | 0.222 |
| *yes* | 9 | 29 | | | |
| *no* | 36 | 45 | | | |
| Substage | | | 2.28 | 1.12-4.66 | 0.023* |
| *b* | 19 | 41 | | | |
| *a* | 26 | 70 | | | |
| Patnaik grade | | | 1.43 | 0.74-2.78 | 0.285 |
| *3* | 20 | 46 | | | |
| *1, 2* | 25 | 41 | | | |
| Kiupel grade | | | 1.31 | 0.66-2.57 | 0.438 |
| *high grade* | 29 | 46 | | | |
| *low grade* | 16 | 36 | | | |
| c-Kit mutations | | | 1.10 | 0.58-2.10 | 0.775 |
| *yes* | 16 | 42 | | | |
| *no* | 29 | 46 | | | |
| Measurable primary tumor at diagnosis of stage IV disease | | | 2.93 | 1.36-6.30 | 0.006* |
| *yes* | 32 | 21 | | | |
| *no* | 13 | 125 | | | |
| Surgery | | | 1.11 | 0.57-2.17 | 0.761 |

| | | | | | |
|---|---|---|---|---|---|
| *no* | 14 | 36 | | | |
| *yes* | 31 | 46 | | | |
| Radiation therapy | | | 1.13 | 0.44-2.92 | 0.797 |
| *no* | 40 | 42 | | | |
| *yes* | 5 | 120 | | | |
| Medical treatment | | | 1.47 | 0.45-4.82 | 0.526 |
| *no* | 3 | 21 | | | |
| *yes* | 42 | 45 | | | |
| Type of medical treatment | | | 1.25 | 0.59-2.64 | 0.529 |
| *TKI only* | 22 | 42 | | | |
| *chemotherapy only* | 7 | 41 | | | |
| *chemotherapy and TKI* | 13 | 90 | | | |
| Use of TKIs in the presence of c-Kit mutations | | | 1.27 | 0.65-2.48 | 0.487 |
| *yes* | 14 | 42 | | | |
| *no* | 31 | 46 | | | |
| Treatment toxicity | | | 1.02 | 0.52-2.02 | 0.944 |
| *yes* | 13 | 81 | | | |
| *no* | 32 | 42 | | | |

485 PFI = progression free interval; HR = hazard ratio; CI = confidence interval; TKI = tyrosine
486 kinase inhibitor; ǂ = median value; * = significant.

487

488 **Table 2** – Univariate Cox regression analysis of variables potentially associated with

489 increased risk of tumor-related death in 45 dogs with stage IV cutaneous mast cell tumors.

| **Variable** | **No. of dogs** | **Median OS** (days) | **HR** | **95% CI** | ***P*** |
|---|---|---|---|---|---|
| Age | | | 1.18 | 0.63-1.18 | 0.609 |
| *> 9 years‡* | 21 | 93 | | | |
| *≤ 9 years* | 24 | 146 | | | |
| Sex | | | 1.00 | 0.54-1.86 | 0.996 |
| *male* | 22 | 109 | | | |
| *female* | 23 | 110 | | | |
| Neutered | | | 1.03 | 0.55-1.93 | 0.922 |
| *no* | 25 | 101 | | | |
| *yes* | 20 | 133 | | | |
| Weight | | | 1.81 | 0.95-3.43 | 0.070 |
| *≤ 25 kg‡* | 23 | 93 | | | |
| *> 25 kg* | 22 | 133 | | | |
| Anatomic site associated with a worse prognosis | | | 1.52 | 0.66-2.28 | 0.521 |
| *yes* | 24 | 101 | | | |
| *no* | 21 | 146 | | | |
| Tumor diameter at presentation | | | 2.43 | 1.27-4.66 | 0.007* |
| *> 3 cm* | 24 | 72 | | | |
| *≤ 3 cm* | 21 | 209 | | | |
| More than 2 metastatic sites | | | 3,30 | 1.43-7.62 | 0.005* |

| | | | | | |
|---|---|---|---|---|---|
| *yes* | 33 | 77 | | | |
| *no* | 12 | 198 | | | |
| Regional lymph node metastasis | | | 38.46 | 1.17-1259.61 | 0.040* |
| *yes* | 41 | 109 | | | |
| *no* | 4 | 940 | | | |
| Bone marrow infiltration | | | 3.88 | 1.69-8.92 | 0.001* |
| *yes* | 9 | 35 | | | |
| *no* | 36 | 146 | | | |
| Substage | | | 2.94 | 1.48-5.85 | 0.002* |
| *b* | 19 | 72 | | | |
| *a* | 26 | 154 | | | |
| Patnaik grade | | | 1.22 | 0.79-2.79 | 0.222 |
| *3* | 20 | 77 | | | |
| *1, 2* | 25 | 146 | | | |
| Kiupel grade | | | 1.30 | 0.73-2.71 | 0.302 |
| *high grade* | 29 | 101 | | | |
| *low grade* | 16 | 150 | | | |
| c-Kit mutations | | | 1.35 | 0.73-2.54 | 0.352 |
| *yes* | 16 | 65 | | | |
| *no* | 29 | 133 | | | |
| Measurable primary tumor at diagnosis of stage IV disease | | | 1.79 | 0.86-3.66 | 0.105 |
| *yes* | 32 | 93 | | | |
| *no* | 13 | 180 | | | |
| Surgery | | | 1.07 | 0.55-2.07 | 0.851 |

| | | | | | |
|---|---|---|---|---|---|
| *no* | 14 | 125 | | | |
| *yes* | 31 | 109 | | | |
| Radiation therapy | | | 1.24 | 0.48-3.19 | 0.659 |
| *no* | 40 | 109 | | | |
| *yes* | 5 | 209 | | | |
| Medical treatment | | | 1.67 | 0.40-6.96 | 0.480 |
| *yes* | 42 | 109 | | | |
| *no* | 3 | 180 | | | |
| Type of medical treatment | | | 1.12 | 0.55-2.30 | 0.633 |
| *TKI only* | 22 | 93 | | | |
| *chemotherapy only* | 7 | 154 | | | |
| *chemotherapy and TKI* | 13 | 146 | | | |
| Use of TKIs in the presence of c-Kit mutations | | | 1.62 | 0.84-3,13 | 0.148 |
| *yes* | 14 | 65 | | | |
| *no* | 31 | 133 | | | |
| Treatment toxicity | | | 1.25 | 0.64-2.44 | 0.523 |
| *yes* | 13 | 110 | | | |
| *no* | 32 | 109 | | | |
| Local tumor control | | | 5.44 | 2.49-11.85 | <0.001* |
| *no* | 13 | 28 | | | |
| *yes* | 32 | 154 | | | |
| Distant tumor control | | | 2.76 | 1.44-5.29 | 0.002* |
| *no* | 22 | 32 | | | |
| *yes* | 23 | 180 | | | |

490

491 OS = overall survival; HR = hazard ratio; CI = confidence interval; TKI = tyrosine kinase

492 inhibitor; ǂ = median value; * = significant.

493

494 **Table 3**. Multivariate Cox regression analysis of variables potentially associated with

495 increased risk of disease progression in 45 dogs with stage IV cutaneous mast cell tumors.

| **Variable** | **HR** | **95% CI** | ***P*** |
|---|---|---|---|
| Tumor diameter > 3 cm at presentation | 3.37 | 1.43-7.97 | 0.006* |
| More than 2 metastatic sites | 3.01 | 1.22-7.40 | 0.017* |
| Regional lymph node metastasis | 631062.63 | 0.00-1.84E223 | 0.958 |
| Substage b | 1.08 | 0.40-2.50 | 0.837 |
| Measurable primary tumor at diagnosis of stage IV disease | 2.47 | 1.08-5.40 | 0.028* |

496 * = significant.

497

**Table 4**. Multivariate Cox regression analysis of variables potentially associated with increased risk of tumor-related death in 45 dogs with stage IV cutaneous mast cell tumors.

| **Variable** | **HR** | **95% CI** | ***P*** |
|---|---|---|---|
| Tumor diameter > 3 cm at presentation | 1.80 | 0.79-4.07 | 0.160 |
| More than 2 metastatic sites | 1.27 | 0.52-3.14 | 0.597 |
| Regional lymph node metastasis | 786953.13 | 0.00-1.19E233 | 0.960 |
| Bone marrow infiltration | 3.43 | 1.30-9.06 | 0.013* |
| Substage b | 1.29 | 0.59-2.84 | 0.520 |
| Lack of local tumor control | 4.51 | 1.54-13.14 | 0.006* |
| Lack of distant tumor control | 1.08 | 0.45-2.58 | 0.856 |

* = significant.