DOI: 10.1002/vms3.812

ORIGINAL ARTICLE

WILEY

Canine mast cell tumours part I: Clinical and survival outcomes

Vanessa S. Tamlin[1] | Cynthia D. K. Bottema[2] | Lucy Woolford[2] | Elizabeth C. Dobson[3] | Allan E. Kessell[4] | Anne E. Peaston[2]

[1]Australian Genome Research Facility Ltd, Melbourne, Victoria, Australia

[2]School of Animal and Veterinary Sciences, The University of Adelaide, Roseworthy, Australia

[3]ASAP Pathology Ltd, Mulgrave, Victoria, Australia

[4]Gribbles Veterinary Pathology, Glenside, Australia

Correspondence
Anne E. Peaston, School of Animal and Veterinary Sciences, The University of Adelaide, Roseworthy, South Australia 5371, Australia.
Email: anne.peaston@adelaide.edu.au

Present address: Vanessa S. Tamlin, Australian Genome Research Facility Ltd, Melbourne, Victoria 3000, Australia.

Elizabeth C. Dobson, ASAP Pathology, Mulgrave, Victoria, 3170, Australia.

Funding information
Australian Companion Animal Health Foundation, Grant/Award Number: 015/2015

Abstract

Background: Dogs have a species-specific susceptibility for developing mast cell tumours (MCTs). Mutations in the *KIT proto-oncogene* (*KIT*) are known to contribute to the neoplastic biology of mast cells. In dogs, the most common *KIT* mutation is an internal tandem duplication (ITD) in exon 11 which has been considered a useful prognostic supplement to traditional histopathological tumour grading.

Objective: The aim of this retrospective study was to explore the importance of *KIT* exon 11 ITD mutation status and known clinical and pathological indices in predicting prognosis in a cohort of Australian dogs diagnosed with MCT.

Methods: Clinical parameters, survival data, and *KIT* mutation status were collected and assessed for 220 dogs with cutaneous or subcutaneous MCT ($n = 189$ and $n = 31$, respectively).

Results: In at least one of the multivariable models, tumour grade (cutaneous Kiupel low or high grade) or tumour subcutaneous location, multiple concurrent MCTs, metastasis at the time of surgery, and senior age were statistically significant in predicting the outcome (MCT-related death and/or second MCT diagnosis) at 6- or 12-month post-tumour excision. *KIT* exon 11 ITD mutation status was not a significant predictor in any of the final multivariable models and was strongly correlated with high histological grade ($p < 0.001$).

Conclusion: In this sample of dogs, tumour histological grading remained the single most powerful prognostic indicator for MCT outcome. However, concurrent evaluation of multiple prognostically significant parameters provides information of potential value to inform therapeutic management for each patient.

KEYWORDS
dog, KIT, mutation, prognosis

1 | INTRODUCTION

Mast cell tumours (MCTs) account for 10%–21% of all canine cutaneous cancers (Tamlin et al., 2020). The biological behaviour of canine cutaneous MCTs varies and is strongly predicted by tumour histopathological grading (Kiupel & Camus, 2019). Two histopathological grading schematics are commonly used in veterinary pathology for canine cutaneous MCTs, the three-tier Patnaik system and the two-tier Kiupel system (Kiupel et al., 2011; Patnaik et al., 1984). In both systems, a higher grade predicts more aggressive tumour biology, and the grading helps to guide surgical and medical management.

MCTs restricted to the subcutaneous fat are termed subcutaneous MCTs. Subcutaneous MCTs occur less frequently than cutaneous tumours and are rarely biologically aggressive (Newman et al., 2007; Thompson, Pearl, et al., 2011). The Patnaik and Kiupel grading systems do not provide useful prognostic information for subcutaneous MCTs.

In addition to tumour histological grade, the prognosis for dogs with MCT can be more accurately predicted by concurrently evaluating patient signalment, clinical signs, and tumour phenotype (Kiupel, 2016; Kiupel & Camus, 2019). The presence of multiple concurrent lesions at diagnosis should be considered in prognosis but does not necessarily indicate more aggressive disease (Tamlin et al., 2020).

Evaluating molecular factors can provide valuable prognostic information for dogs with MCT (Kiupel, 2016; Kiupel & Camus, 2019). One such factor is mutation within the *KIT proto-oncogene* (*KIT*) (Thamm et al., 2019). The *KIT* proto-oncogene encodes Kit, a transmembrane tyrosine kinase receptor protein involved in the development, proliferation, and function of mast cells, melanocytes, interstitial cells of Cajal, and haematopoietic stem cells (Lennartsson et al., 2005). Kit protein is normally activated by binding its ligand, haematopoietic stem cell factor (SCF). This causes Kit dimerisation and phosphorylation, triggering downstream intracellular signalling cascades inducing mast cell development, survival, proliferation, secretory function, and chemotaxis (Lennartsson et al., 2005;Letard et al., 2008). Gain-of-function mutations within the *KIT* gene disrupt normal Kit protein function, leading to constitutive Kit activation in the absence of ligand binding and uncontrolled neoplastic mast cell proliferation (Furitsu et al., 1993; Letard et al., 2008; Nakano et al., 2017). Approximately 18% of cutaneous canine MCTs carry an exon 11 internal tandem duplication (ITD) activating mutation, and the frequency of *KIT* exon 11 ITD increases with increasing tumour histological grade (Downing et al., 2002; Horta et al., 2018; Tamlin et al., 2017).

Until recently, *KIT* mutations in subcutaneous MCTs had not been reported (Tamlin et al., 2019; Vozdova et al., 2020). A preliminary Australian study reported a *KIT* exon 11 ITD frequency of 10% in cutaneous canine MCTs ($n = 24/239$) and a 2% frequency in subcutaneous MCTs ($n = 1/41$) (Tamlin et al., 2019). This molecular work provided the basis for the current investigation. The primary objective herein was to evaluate the relationships between survival outcomes of dogs with MCT and canine clinical parameters, tumour phenotype, and *KIT* exon 11 ITD status. It was hypothesised that tumour *KIT* exon 11 ITD status would not add any additional prognostic information to that achieved by tumour histological grade.

2 | MATERIALS AND METHODS

2.1 | Data collection

A total of 291 formalin-fixed, paraffin-embedded (FFPE) canine MCT blocks collected for a previous investigation provided the basis for the current study (Tamlin et al., 2019). Briefly, the tumours were submitted to veterinary pathology laboratories from primary veterinary practices in south and south-eastern Australia. DNA from all 291 FFPE tumour specimens and from fresh-frozen normal canine testes was extracted using a commercial kit (QIAamp DNA FFPE Tissue kit; Qiagen, Hilden, Germany). The DNA samples were tested for their ability to be amplified by PCR and for the presence of a *KIT* exon 11 ITD according to previously described methods (Tamlin et al., 2019). *KIT* exon 11 ITD mutation status for each tumour was recorded.

The patient clinical data were obtained from the MCT histopathological reports held in the databases at three veterinary pathology laboratories (Gribbles Veterinary Pathology Laboratories Glenside SA and Clayton VIC, and the Veterinary Diagnostic Laboratory, School of Animal and Veterinary Sciences, University of Adelaide SA). Briefly, these data included the signalment, number of concurrent MCTs, tumour anatomical location, tumour size (≤30 mm or >30 mm based on the largest measured diameter) (Mullins et al., 2006; O'Connell & Thomson, 2013; Pierini et al., 2019), completeness of tumour excision, and primary veterinary practice geographical location (metropolitan or rural area).

Patient history and survival data encompassing MCT treatment, second MCT diagnosis, either cytologically or histologically confirmed tumour metastasis at the time of tumour excision, previous history of MCT, health status at final follow-up/cause of death, and any patient signalment data missing from the pathology reports were collected through telephone and email contact with the veterinary staff at primary practices. A primary veterinary practice was defined as the clinic which excised the MCT and submitted it to the pathology laboratory.

To determine tumour histological grade, at least one haematoxylin and eosin-stained section of each MCT was graded independently by two or more board-certified veterinary pathologists. Each tumour was classified as cutaneous or subcutaneous, and cutaneous tumours were graded as Kiupel low or high grade (Kiupel et al., 2011). The diagnosis of a subcutaneous MCT was based on the tumour being located completely within the subcutis and no invasion of the dermis or epidermis. All pathologists graded blindly. Where grades were discordant, the results were unblinded and the pathologists re-examined and discussed the same sections until a unanimous decision was made. If an agreement could still not be made, the highest histological grade was assigned to the tumour to account for the worst-case scenario. Mucocutaneous MCTs were not collected for this study.

2.2 | Survival analyses

For dogs with more than one concurrent MCT, the tumour of the highest histological grade for that dog was recorded for the survival analyses. In instances where a dog was diagnosed with both cutaneous and subcutaneous MCTs, survival data regarding the cutaneous lesion were recorded as a cutaneous tumour is considered to have a poorer prognosis (Newman et al., 2007; Thompson, Pearl, et al., 2011).

For the survival analyses, dogs were grouped according to the Australian National Kennel Council's (ANKC) recognised breed groups, rather than as individual breeds, to ensure a sufficient number of animals were in each breed group to allow for a statistically significant model. Dogs were grouped as either 'adult' or 'senior' based on their age and their breed size to account for the different average life expectancies of different breed sizes (Greer et al., 2007). A dog was classified as senior if ≥10 years old and small (average breed weight 1–10 kg), ≥8 years old and medium (average breed weight 11–26 kg), ≥7 years old and large (average breed weight 27–44 kg), or ≥6 years old and giant (average breed weight ≥45 kg).

Survival was measured in days from the date of tumour surgical excision to the date of death. Dogs lost to follow-up, healthy dogs alive at the end of follow-up, and dogs which died or were euthanised due to non-MCT related causes were right censored from the survival analyses. To avoid underestimation of disease-related morbidity and mortality, dogs with an unknown health status at final follow-up were grouped with dogs diagnosed with a second MCT, and dogs with an unknown cause of death were grouped with those whose death was MCT related. To examine the possibility that this would overestimate MCT-related morbidity and mortality, a similar analysis was conducted in which dogs with an unknown health status at final follow-up or dogs with an unknown cause of death were right censored. Median time to MCT-related death was defined as the time (in days) at which 50% of dogs had died due to MCT.

A lack of detailed clinical data available from the primary practices regarding disease progression prevented an accurate calculation of tumour-free survival (TFS) or progression-free interval (PFI). Instead, time to a second MCT diagnosis was determined to be a suitable substitute for measuring TFS in this study. Time to a second MCT diagnosis was measured in days from the date of surgery until the date of second MCT diagnosis, providing the diagnosis was confirmed by histopathology and not cytology alone. The median time to the second MCT diagnosis was defined as the time (in days) at which 50% of all dogs that developed a second MCT were diagnosed.

A history of MCT diagnosis prior to the tumour collected for this study was determined from clinical records. Dogs with a history of prior MCT diagnosis were not considered cases of multiple MCT. Only dogs with ≥2 concurrent MCT lesions at the time of surgery were considered cases of multiple MCT.

2.3 | Statistical analyses

All statistical analyses were performed in IBM SPSS statistical software (version 25, Armonk). Statistical significance was established at $\alpha < 0.05$. A generalised binary logistic regression model was performed to ascertain statistically significant differences in exon 11 *KIT* ITD mutation prevalence between patient clinical variables and survival data. Wald chi-square analysis was used to test for statistical significance.

Univariable and multivariable Cox proportional hazards regression models were used to assess the influence of the clinical variables on 6- and 12-month canine MCT-related deaths and second MCT diagnosis. Variables having a p-value < 0.25 in the univariable analyses were included in the multivariable model building (Bursac et al., 2008). Using backward selection methods, only those variables that were significant at a level of $p < 0.05$ were retained in the multivariable models, except for tumour type (cutaneous Kiupel low- or high-grade MCT or subcutaneous MCT). Tumour type was included in all multivariable models, regardless of statistical significance, because distinguishing subcutaneous tumours from cutaneous tumours is prognostically important (Newman et al., 2007; Thompson, Pearl, et al., 2011). Cases with missing data were omitted from statistical analyses. Survival outcomes were estimated using the Kaplan–Meier Estimator of Survival Probability and compared by the log-rank test (outcomes being time to MCT-related death or second MCT diagnosis).

Pearson chi-square analysis (χ^2) was used to evaluate the frequency distributions of tumour histological grade and breed in adult and senior animals, and to determine the histological grade frequencies between breeds represented by more than 10 animals with cutaneous MCT. Cellwise residual analysis on two-way contingency table method was used to establish which demographic group was statistically different from the population cohort (Beasley & Schumacker, 1995; García-pérez & Núñez-antón, 2016). p-Values were adjusted to minimise type-I errors (false-positives) as recommended and previously described (Beasley & Schumacker, 1995). Dogs with subcutaneous MCTs were not considered in this analysis due to a limited number of individuals with subcutaneous MCT when segregated by breed.

3 | RESULTS

3.1 | Evaluable cases

Of the 291 FFPE blocks submitted for this study, 280 were histologically confirmed cutaneous or subcutaneous MCTs and were sourced from 248 dogs visiting 130 primary veterinary practices throughout south and south-eastern Australia. Clinical data were collected from 118 (90.8%) of the 130 primary practices upon agreement to participate in the study. Data were not collected from the remaining 12 clinics due to computer system updates at the clinic resulting in loss of patient records ($n = 8$), patient-doctor confidentiality restrictions ($n = 2$), non-consenting pet owners ($n = 1$), or close of practice ($n = 1$). Consequently, clinical follow-up data were unavailable for 24 animals. Follow-up data were unavailable for a further four dogs which were not seen at their primary practice after tumour excision. These dogs were excluded from the survival analyses. Overall, clinical and survival data were available for 220 dogs.

TABLE 1 Population demographics for 220 dogs with cutaneous or subcutaneous mast cell tumours (MCTs)

Breed[a]	Count (%)
Crossbreed	57 (25.9)
Terrier	57 (25.9)
Gun dog	52 (23.6)
Other	29 (13.2)
Utility dog	25 (11.4)
Multiple MCTs	
Single	200 (91.9)
Multiple	20 (9.09)
Age	
Senior	141 (64.1)
Adult	79 (35.9)
Sex and neuter status	
Female spayed	106 (48.2)
Female entire	20 (9.09)
Male entire	29 (13.2)
Male castrated	65 (11.4)
Tumour anatomical location	
Trunk	84 (38.2)
Limb	60 (27.3)
Multiple locations	20 (9.09)
Other	25 (11.4)
Head/neck	18 (8.18)
Paw	8 (3.64)
Tail	5 (2.27)
Tumour size	
≤30 mm in diameter	179 (81.4)
>30 mm in diameter	41 (18.6)
Geographical location	
Metropolitan	137 (62.3)
Rural	83 (37.7)
History of MCT	
No	208 (94.6)
Yes	12 (5.45)
Health status at last follow-up	
Alive, without MCT	128 (58.2)
Alive, with MCT	18 (8.18)
Died/euthanised, MCT-related	46 (20.9)
Died/euthanised, MCT-unrelated	28 (12.7)
Post-surgical treatment	
None	198 (90.0)
Cytotoxic chemotherapy	8 (3.64)
Radiotherapy	5 (2.27)
TKI	4 (1.82)
Chemotherapy with TKI	3 (1.36)
Chemotherapy with radiotherapy	2 (0.91)

Abbreviation: TKI, tyrosine kinase inhibitor.

[a]Breeds in this study represented in the ANKC Terrier groups: Staffordshire Bull Terrier (31), Jack Russell Terrier (21), Fox Terrier (2), Tenterfield Terrier (2), Bull Terrier (1); Gun dog group: Labrador Retriever (37), Golden Retriever (14), German Shepard (1); Other group: Pug (12), Bulldog (5), Australian Cattle dog (1), Australian Shetland Sheepdog (1), Beagle (1), Boston Terrier (1), Bull Arab (1), Chihuahua (1), French Bulldog (1), Kelpie (1), Lhasa Apso (1), Maltese (1), Poodle (1), Rhodesian Ridgeback (1); and Utility dog group: Boxer (20), Bernese Mountain dog (2), Bull Mastiff (1), Miniature Schnauzer (1), and Saint Bernard (1).

3.2 | Population demographics

The mean age of the 220 dogs was 8.72 ± 2.79 years (range 3–17 years). Twenty dogs were diagnosed with multiple concurrent MCTs at the time of surgery; one dog had four cutaneous tumours, three dogs had three cutaneous tumours, 15 dogs had two cutaneous tumours, and one dog had one cutaneous tumour and one subcutaneous tumour (Table 1). Tumours ranged from 3 to 100 mm in diameter (mean size of 21.0 ± 16.5 mm). Twelve dogs had a history of previous MCT diagnosis and the dates for previous tumour diagnosis were known for eight of these dogs. For these eight dogs, the mean and median time between historically recorded MCT and the MCT recorded in the current study was 1171 ± 638 days and 1142 days, respectively (range 344–2434 days). Insufficient data were available to ascertain whether the recently excised tumour represented a de novo neoplasm or recurrence of the historically recorded MCT. A *KIT* exon 11 ITD was detected in 9.55% of dogs with MCT (n = 21/220, 95% confidence interval [CI]: 5.66–13.4) (Table 2).

3.3 | Clinical outcomes

The median and mean post-excision follow-up time for the 220 dogs was 548 days and 598 days, respectively. The shortest follow-up time was recorded as two days for 2 dogs. One of these dogs was seen for wound re-bandaging and not again thereafter, and the other dog was diagnosed with systemic hyperhistaminemia and subsequently died.

Over the study period, 28/220 dogs (12.7%) died for reasons unrelated to MCT, 27 dogs (12.3%) experienced documented MCT-related death and an additional 19 dogs (8.6%) died or were euthanised for unknown reasons. Thus, MCT accounted for or potentially accounted for 46 deaths (20.9%). Over the same period, 56 dogs had a second cutaneous MCT diagnosed and three dogs were of unknown MCT status at last follow-up. Thus, 59 dogs (26.8%) developed or potentially developed a second MCT. The median times to MCT-related death for the 46 dogs and second MCT diagnosis for the 59 dogs were > 3272 (range 2–3272) days and 1348 (range 2–3272) days, respectively. The same results were obtained when dogs were censored from

TABLE 2 Tumour type (cutaneous Kiupel low- or high-grade mast cell tumour (MCT) and subcutaneous MCT) with selected key clinical and molecular features for 220 dogs diagnosed with MCT

Tumour type	Low grade	High grade	Subcutaneous	Total
Count (%)	144 (65.5)	45 (20.5)	31 (14.1)	220 (100)
Incomplete margins	55 (38.2)	26 (57.8)	15 (48.3)	96 (43.6)
Metastasis at surgery	2 (1.39)	8 (17.8)	0 (0.00)	10 (4.55)
Second diagnosis of MCT	37 (25.7)	16 (35.6)	6 (19.4)	59 (26.8)
KIT exon 11 ITD	1 (0.69)	19 (42.2)	1 (3.23)	21 (9.55)

Abbreviation: ITD, internal tandem duplication.

TABLE 3 Mast cell tumour (MCT)-related death and second MCT diagnosis by tumour type (cutaneous Kiupel low- or high-grade MCT and subcutaneous MCT). At final follow-up of the 220 dogs, 46 dogs died due to MCT and 59 dogs were diagnosed with a second MCT

Event	Time to event	Total	Low grade	High grade	Subcutaneous	*p*-Value
MCT-related death	6 months (%)	10.5	4.58[a]	30.4[a]	10.4	<0.001
	12 months (%)	15.6	6.22[a]	50.3[ab]	14.4[b]	<0.001
	24 months (%)	21.5	9.01[a]	75.4 [ab]	19.2[b]	<0.001
Second MCT diagnosis	6 months (%)	12.9	10.5[a]	27.6[ab]	3.8[b]	0.008
	12 months (%)	19	15.6[a]	42.1[ab]	8.7[b]	0.001
	24 months (%)	31.7	26.3[a]	66.9[ab]	33.7[b]	0.002

[a]Statistically significant difference indicated between dogs with low- and high-grade MCTs.
[b]Statistically significant difference indicated between dogs with subcutaneous MCTs and high-grade MCTs.

analyses because of unknown cause of death or unknown health status at final follow-up. Of the 56 dogs diagnosed with a second MCT, seven were diagnosed >24 months post-initial tumour excision. MCT-related death rates and the frequency of second MCT diagnosis at 6-, 12- and 24-month post-tumour excision were statistically significantly different between dogs with tumours of different histological grades (Table 3 and Figure 1).

3.4 | Predicted MCT-related deaths and diagnosis of a second MCT

In the univariable analyses for the 220 dogs with MCT, significant predictors for 6- and/or 12-month canine MCT-related deaths included *KIT* exon 11 ITD mutation status, tumour type, age, incomplete tumour excision, metastasis at the time of surgery, and the diagnosis of a second MCT (Table 4). Similarly, significant predictors for second MCT diagnosis at 6 and 12 months were *KIT* exon 11 ITD mutation status, tumour type, multiple MCTs, and metastasis at the time of surgery.

In the multivariable analyses, tumour type, senior age, and confirmed metastasis at the time of surgery were statistically significant risk factors for predicting 6- and 12-month MCT-related deaths (Table 5). *KIT* exon 11 ITD mutations were not significant in the final multivariable model ($p = 0.667$), despite a strong relationship with 12-month MCT-related deaths predicted in the univariable analysis ($p < 0.001$).

Significant risk factors for a second MCT diagnosis at 6 months in the multivariable analysis included tumour type and the presence of multiple concurrent MCTs at initial diagnosis (Table 5). At 12 months, tumour type (cutaneous Kiupel high-grade), multiple MCTs and confirmed metastasis at the time of surgery were significant predictors. To determine whether the low rate of *KIT* exon 11 ITDs in subcutaneous MCTs skewed the data, the multivariable analyses were repeated excluding subcutaneous MCT cases. The same statistical outcomes were reached whereby the *KIT* exon 11 ITD status was not a significant predictor of MCT-related death or second MCT diagnosis at 6 or 12 month post-surgery.

3.5 | Relationships among histological grade, age, and breed

The proportion of senior dogs with MCTs was higher in Labrador Retrievers (90.3%, $n = 28/31$, $p < 0.001$) and lower in Pugs (16.7%, $n = 2/12$, $p < 0.001$) (Table 6). The frequency of high-grade MCTs was higher for Labrador Retrievers (45.2%, $n = 14/31$, $p = 0.002$) and 100% of Pugs presented with low-grade MCT. Overall, a higher prevalence of high-grade MCTs was observed in senior animals compared to adult animals, 32.8% ($n = 39/119$) versus 8.57% ($n = 6/70$), respectively ($p < 0.001$).

3.6 | *KIT* exon 11 ITD correlations with prognostic indices

In the univariable analysis, MCT *KIT* exon 11 ITD prevalence was significantly correlated with high histological tumour grade ($p < 0.001$),

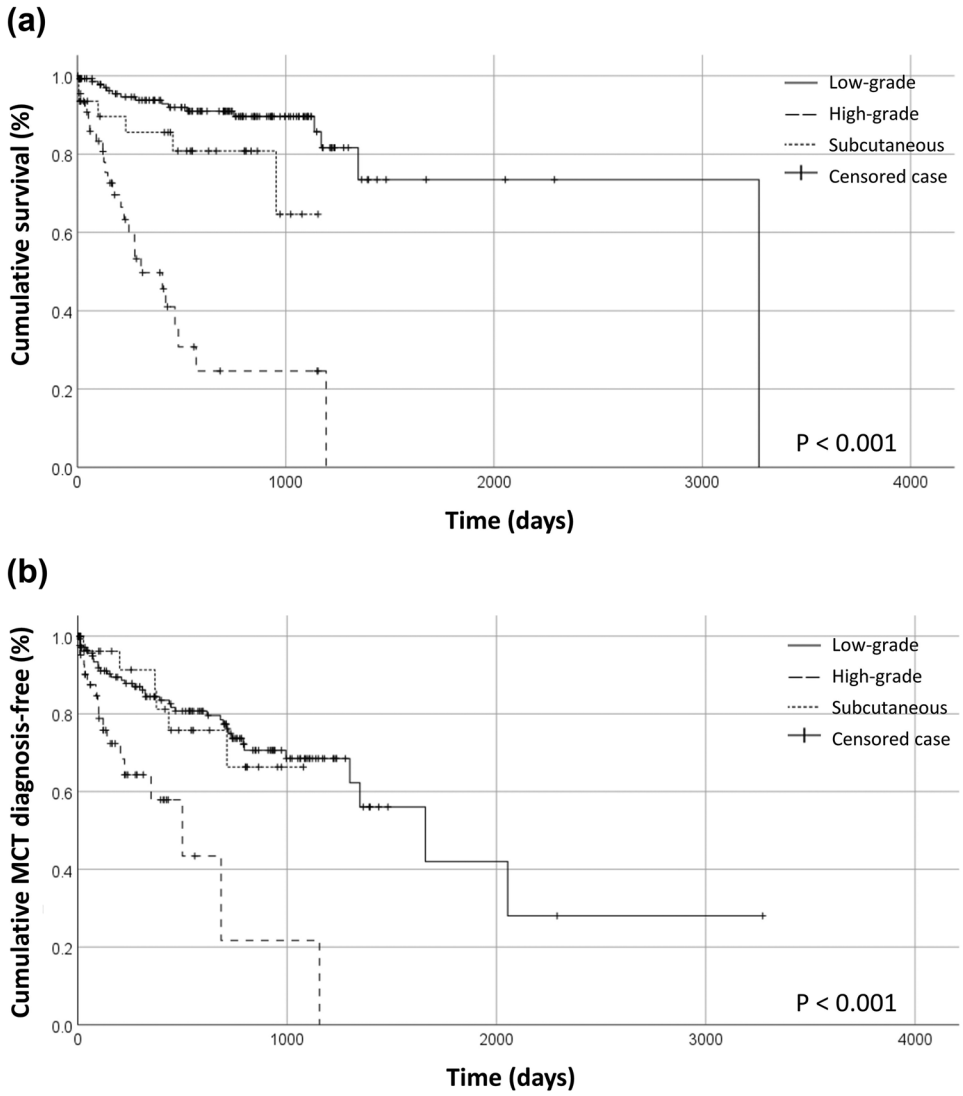

FIGURE 1 Kaplan–Meier curves for (a) mast cell tumour (MCT)-related survival and (b) MCT-free diagnosis, according to tumour type in 220 dogs with low-grade, high-grade, or subcutaneous MCT

incomplete tumour excision ($p = 0.031$), and confirmed metastasis at the time of surgery ($p < 0.001$). In the final multivariable model, tumour type and confirmed metastasis at the time of surgery were statistically significant in predicting the likelihood that MCTs would have a *KIT* exon 11 ITD (χ^2 [3] = 64.862, $p < 0.001$). The multivariable model explained 54.6% (Nagelkerke R^2) of the variance in ITD mutation frequency of the data and correctly classified 93.2% of cases. Kiupel high-grade tumours were 80.1 times more likely to have a *KIT* exon 11 ITD than Kiupel low-grade tumours in the multivariable model (95% CI: 10.0–640, $p < 0.001$), whereas there was no statistically significant difference in ITD frequency between low-grade and subcutaneous MCTs ($p = 0.237$). Confirmed metastasis at the time of surgery was associated with a 12.3 times increased risk of having a *KIT* exon 11 ITD (95% CI: 1.71–88.8, $p = 0.013$).

4 | DISCUSSION

This is the largest retrospective clinical and survival study of canine MCTs to be published from Australian data. In univariable survival analyses of 220 dogs with cutaneous or subcutaneous MCT, dogs with a *KIT* exon 11 ITD-mutant tumour had over twice the risk of dogs with non-ITD-mutant tumours for MCT-related death and second MCT diagnosis at 6 months (Table 3). After accounting for tumour type in the multivariable survival models, the statistical significance of this effect was lost (Table 5). These findings are in agreement with a recent report showing that in a multivariable analysis of a group of 149 dogs, *KIT* exon 11 ITD mutation was not independent of histological grade as a statistically significant predictor of overall survival (Horta et al., 2018). Two older studies reported that a *KIT* exon 11 ITD was significant

in multivariable analysis for predicting shorter overall canine survival and disease-free intervals, but these studies had low sample numbers (Webster et al., 2008) or failed to include histological grade in the analysis (Webster et al., 2006). Therefore, the prognostic value of *KIT* exon 11 ITD mutation status independent of histological grade is debatable.

Consistent with previous reports, the data herein indicate that dogs with a cutaneous high-grade MCT have a significantly decreased chance of survival at 6, 12 and 24 months post-surgery compared to dogs with a cutaneous low-grade or subcutaneous MCT (Kiupel et al., 2011; Murphy et al., 2004; Newman et al., 2007; Thompson, Pearl, et al., 2011; Thompson, Yager, et al., 2011). Dogs with subcutaneous MCTs had similar survival rates to dogs with cutaneous low-grade MCTs.

Herein, a statistically significantly higher prevalence of high-grade cutaneous MCTs was diagnosed in senior animals compared to adult animals, 32.8% versus 8.57% respectively, suggesting that senior dogs experience more aggressive disease (Mochizuki et al., 2017; Smiech et al., 2018). An explanation for this is unclear but may be a consequence of selection bias. Pet owners and veterinarians may be less motivated to investigate benign-appearing lumps in senior dogs, potentially leading to under-diagnosis of low-grade MCT and consequent inflation of the prevalence of high-grade MCT in older animals. Alternatively, the significance of age in the univariable survival model may be a result of the predisposition of particular dog breeds for developing MCTs of different malignant phenotypes at different ages. For example, 90% of Labrador Retriever dogs in this study were classified as senior (≥7 years old) and 45.2% had high-grade MCTs, whereas 83.3% of Pugs were classified as adults (<10 years old) and all had low-grade MCTs. No other correlations with age, breed and histological tumour grade were observed.

Breeds reported to be susceptible to MCT development vary considerably. Those commonly regarded as being predisposed to MCTs include breeds of bulldog origin (American and English Staffordshire Bull Terriers, Boston Terriers, Boxers, French and English Bulldogs, and Pugs), Shar Pei dogs, Golden Retrievers, and Labrador Retrievers (Jaensch, 2008; Leidinger et al., 2014; Mochizuki et al., 2017; Smiech et al., 2019; Warland & Dobson, 2013). In this study, Labrador Retrievers were at a statistically significant risk for high-grade MCT diagnosis at an older age (≥7 years old). Conversely, two studies by the same group found that Labradors in Poland were at risk for low-grade MCT development at a younger age, although it is notable that more than 80% of Labrador MCTs were low grade in these studies (4–6 years; $p = 0.006$) (Smiech et al., 2019, 2018). In one recent Australian study, 31.3% of MCTs from Labradors were diagnosed as high-grade tumours ($n = 10/32$), although age was not considered in the analysis and the hazard for high-grade MCT development in Labradors was not significant (Reynolds et al., 2019). The discrepancies in Labrador-related risks for MCT development may reflect the geographical differences in MCT genetics in relatively closed canine populations throughout the world. A genome-wide association study in Golden Retrievers from Europe and the United States identified distinct differences in predisposing germ-line genetic factors associated with cutaneous MCT development in the two populations (Arendt et al., 2015). Additionally, the *KIT* exon 11 ITD mutation prevalence in canine MCT populations studied in Europe is substantially lower than the estimated prevalence of mutations in canine populations from American studies (Downing et al., 2002; Giantin et al., 2012; London et al., 1999; Reguera et al., 2002; Riva et al., 2005; Webster et al., 2006). However, small sample sizes or selection bias may confound the results from some studies, and hence, these data should be interpreted cautiously. Additional research on unbiased larger canine populations is required to explore ostensible continental differences in MCT genetics.

Aligning with a previous report, dogs with tumours located on the head/neck or paw had a statistically significant increased risk of 12-month MCT-related death compared to animals with tumours on the trunk in the univariable analysis (Table 4) (Kiupel et al., 2005). Dogs with a head/neck tumour were also at a statistically significant increased risk to be diagnosed with a second MCT at 12 months post-surgery. The reason for this outcome is obscure and did not remain significant in the multivariable model.

Only 9% of animals in this study presented with multiple concurrent tumours ($n = 20/220$), however, up to 21% of all MCT cases have been documented with multiple lesions (Murphy et al., 2006; Pierini et al., 2019). There is a debate in the literature regarding the prognostic utility of the presence of multiple MCT lesions (Tamlin et al., 2020). In the current study, multiple MCTs did not predict decreased survival, although they were a risk factor for a diagnosis of a second MCT at 6 and 12 months in both the univariable and multivariable models (Tables 4 and 5).

At the time of tumour excision, lymph node metastasis was confirmed in 10 dogs with cutaneous MCTs, with a higher frequency of metastasis in dogs diagnosed with a high-grade MCT (17.8%, $n = 8/45$) compared with dogs diagnosed with a low-grade MCT (1.39%, $n = 2/144$) (Table 2). Metastasis was not confirmed in any dogs with subcutaneous MCT, reflecting the favourable prognosis for dogs with subcutaneous tumours (Thompson, Pearl, et al., 2011). Lymph node metastasis has been recorded in 15% of dogs with multiple cutaneous lesions and in up to 19% of dogs with a single cutaneous mass ($n = 8/54$ and $n = 72/386$, respectively) (Mullins et al., 2006; Stefanello et al., 2015). However, these findings are from the retrospective analyses of records of a (anonymised) veterinary teaching hospital and a referral veterinary oncology hospital, whose databases are inherently biased towards the inclusion of more malignant cases. In the current investigation, many patient records failed to mention the state of local lymph nodes and the parameters used for cytological or histological confirmation of lymph node metastasis were unspecified, thus limiting the interpretation of these results. The true rate of metastasis is suspected to be higher than reported here, but lower than that deduced from the analyses of referral databases.

Dogs with confirmed metastasis at the time of surgery were 4 times and 2.95 times more at risk of experiencing 12-month MCT-related death or a second MCT diagnosis, respectively, than dogs without confirmed metastasis as determined by multivariable analysis (Table 5). This finding supports reports of poorer prognosis for dogs with histologically diagnosed metastasis to regional lymph nodes in which the 2-year survival rate post-lymphadenectomy was 56% for dogs with and 90% for dogs without lymph node metastasis (Weishaar et al., 2014). Lymphadenectomy in dogs with lymph node metastasis has been

TABLE 4 Univariable hazard ratios (HR) of prognostic factors for 6- and 12-month mast cell tumour (MCT)-related death and second MCT diagnosis for 220 dogs with cutaneous Kiupel low- or high-grade MCT or subcutaneous MCT

	MCT-related death						Second MCT diagnosis					
	6 months			12 months			6 months			12 months		
Covariate	HR	95% CI	*p*-Value	HR	95% CI	*p*-Value	HR	95% CI	*p*-Value	HR	95% CI	*p*-Value
KIT exon 11 ITD	5.58	2.25–13.9	<0.001*	8.49	4.07–17.7	<0.001*	2.81	1.05–7.50	0.039*	3.28	1.42–7.55	0.005*
Tumour type[a]			0.001*			<0.001*			0.015*			0.008*
Cutaneous Kiupel low-grade[b]	0.000			0.000			0.000			0.000		
Cutaneous Kiupel high-grade	7.68	2.88–20.5	<0.001*	10.0	4.34–23.2	<0.001*	2.87	1.27–6.48	0.011*	2.81	1.36–5.78	0.005*
Subcutaneous	2.65	0.662–10.6	0.168	2.67	0.802–8.85	0.109	0.376	0.049–2.86	0.345	0.537	0.126–2.30	0.402
Multiple MCTs	1.09	0.254–4.68	0.907	0.727	0.173–3.05	0.648	3.6	1.44–9.03	0.006*	3.00	1.31–6.90	0.010*
Breed[a]			0.308			0.170			0.736			0.394
Crossbreed[b]	0.000			0.000			0.000			0.000		
Gun dog	3.39	0.916–12.5	0.067	2.84	1.00–8.07	0.050	1.70	0.480–6.03	0.410	1.02	0.344–3.05	0.967
Other	1.20	0.201–7.20	0.840	0.709	0.138–3.66	0.682	1.37	0.308–6.14	0.677	1.05	0.306–3.57	0.945
Terrier	1.64	0.391–6.85	0.500	1.38	0.438–4.35	0.582	2.26	0.697–7.35	0.174	2.06	0.831–5.10	0.119
Utility dog	1.51	0.252–9.03	0.652	2.84	0.504–7.00	0.348	1.72	0.385–7.68	0.478	1.01	0.262–3.92	0.984
Senior age	11.6	1.56–86.8	0.017*	8.51	2.03–35.7	0.003*	1.26	0.546–2.93	0.584	1.14	0.562–2.30	0.723
Sex and neuter status[a]			0.770			0.479			0.956			0.783
Female entire[b]	0.000			0.000			0.000			0.000		
Female spayed	1.58	0.565–4.44	0.382	1.37	0.595–3.15	0.461	1.06	0.415–2.68	0.910	0.980	0.445–2.16	0.961
Male entire[c]	–	–	–	0.303	0.038–2.42	0.261	1.39	0.408–4.76	0.597	1.46	0.529–4.00	0.468
Male castrated	2.03	0.485–8.50	0.332	1.29	0.342–4.87	0.706	0.994	0.206–4.79	0.994	0.693	0.152–3.16	0.636
Tumour anatomical location[a]			0.176			0.052			0.138			0.098
Trunk[b]	0.000			0.000			0.000			0.000		
Head/neck	2.00	0.517–7.73	0.316	3.45	1.20–9.95	0.022*	2.57	0.643–10.3	0.182	3.13	1.02-9.58	0.045*
Limb	0.781	0.229–2.67	0.693	1.37	0.513–3.65	0.531	1.63	0.548–4.86	0.379	1.74	0.688–4.42	0.241
Multiple	1.23	0.256–5.94	0.794	1.06	0.225–4.99	0.941	4.83	1.56–15.0	0.006*	4.27	1.55–11.8	0.005*
Other	0.472	0.058–3.84	0.483	0.410	0.051–3.28	0.400	0.553	0.067–4.59	0.583	0.803	0.171–3.78	0.782
Paw	5.15	1.33–19.9	0.018*	4.77	1.26–18.0	0.021*	2.12	0.255–17.6	0.487	1.64	0.205–13.1	0.641
Tail	2.05	0.252–16.7	0.502	3.68	0.781–17.3	0.099	2.36	0.284–19.6	0.426	1.80	0.224–14.5	0.581
Tumour size(>30 mm)	1.52	0.558–4.16	0.411	1.57	0.675–3.67	0.294	1.19	0.447–3.18	0.726	0.867	0.335–2.24	0.769
Incomplete tumour excision	1.37	0.588–3.26	0.455	2.17	1.03–4.57	0.041*	1.38	0.631–3.03	0.418	1.82	0.971–3.60	0.087
Geographical location	1.09	0.450–2.62	0.853	1.39	0.673–2.85	0.377	0.969	0.428–2.19	0.940	1.10	0.549–2.19	0.793
Metastasis at time of surgery	7.19	2.63–19.7	<0.001*	10.4	4.56–23.5	<0.001*	4.37	1.50–12.8	0.007*	4.50	1.73–11.7	0.002*
Second diagnosis of MCT	1.57	0.649–3.78	0.318	2.27	1.11–4.64	0.025*	–	–	–	–	–	–
History of MCT	0.774	0.104–5.77	0.803	1.10	0.263–4.63	0.894	0.658	0.089–4.87	0.682	0.467	0.064–3.41	0.453

Abbreviations: CI, confidence interval; ITD, internal tandem duplication.

Variables were considered statistically significant at a p-value < 0.050 (*) using a univariable Cox proportional hazards regression analysis.

[a]For categorical variables with ≥3 sub-groups, a p-value is calculated to imply the overall statistical value of the categorical variable. The sub-groups are then analysed independently against a reference group and HRs can be calculated.

[b]Reference group for analysis indicated by HRs of 0.000.

[c]Zero intact males at 6 months had experienced MCT-related death so the statistical comparison was not possible.

TABLE 5 Multivariable hazard ratios (HR) of prognostic factors for 6- and 12-month mast cell tumour (MCT)-related death and second MCT diagnosis for 220 dogs with cutaneous or subcutaneous MCTs

	MCT-related death			Second MCT diagnosis		
Covariate	**HR**	**95% CI**	***p*-Value**	**HR**	**95% CI**	***p*-Value**
6 months						
Tumour type[a]			0.043*			0.018*
Cutaneous Kiupel low-grade[b]	0.000			0.000		
Cutaneous Kiupel high-grade	3.89	1.34–11.3	0.013*	2.91	1.29–6.57	0.010*
Subcutaneous	2.43	0.606–9.75	0.210	0.458	0.059–3.53	0.454
Multiple MCTs			N/A	3.31	1.31–8.35	0.011*
Senior age	7.71	1.01–59.0	0.049*			N/A
Metastasis at time of surgery	3.55	1.17–10.8	0.025*			N/A
12 months						
Tumour type[a]			0.003*			0.095
Cutaneous Kiupel low-grade[b]	0.000			0.000		
Cutaneous Kiupel high-grade	4.99	2.00–12.4	< 0.001*	2.24	1.00–4.98	0.047*
Subcutaneous	2.47	0.742–8.22	0.141	0.655	0.151–2.84	0.572
Multiple MCTs			N/A	3.04	1.30–7.09	0.017*
Senior age	5.83	1.36–25.1	0.018*			N/A
Metastasis at time of surgery	5.11	2.04–12.8	<0.001*	2.95	1.01–8.56	0.047*

Note: N/A: not included in the model.
[a]For categorical variables with ≥3 sub-groups, a *p*-value is calculated to imply the overall statistical value of the categorical variable. The sub-groups are then analysed independently against a reference group and HRs can be calculated.
[b]Reference group for analysis indicated by the HRs of 0.000.

TABLE 6 Breed demographics for 189 dogs with cutaneous mast cell tumour used for analysing the relationship between canine breed, age (years), and histological grade

Breed group	**Number of senior dogs (%)**	**Age considered to be senior**
Crossbreed	30/49 (61.2)	Dependant on breed size
Boxer	11/18 (61.1)	≥7 years
Golden Retriever	8/14 (57.1)	≥7 years
Jack Russell Terrier	12/18 (66.7)	≥10 years
Labrador Retriever	28/31 (90.3)	≥7 years
Other	10/20 (50.0)	Dependant on breed size
Pug	2/12 (16.7)	≥10 years
Staffordshire Bull Terrier	17/27 (63.0)	≥8 years

associated with a statistically significant increased probability of 12-month survival (94% in 35 dogs with versus 78% in 20 dogs without lymphadenectomy) and with a statistically significantly longer median time to MCT progression (median time not reached in 81 dogs with versus 170 days in 71 dogs without lymphadenectomy) (Baginski et al., 2014; Marconato et al., 2018).

In the current study, no statistically significant associations were discovered between *KIT* exon 11 ITD status and breed, sex, or neuter status. No correlations were observed between the risk of MCT-related death or second MCT diagnosis and sex, neuter status, tumour size, or incomplete tumour excision. Nonetheless, the use of these indicators in prognosis is evident in some literature, and they should be not be overlooked (Kiupel, 2016; Pierini et al., 2019; White et al., 2011).

Other prognostic variables important in mast cell malignancy and canine MCT prognosis include immunohistochemical evaluation of the Kit protein staining pattern and assessment of the proliferative markers AgNOR and Ki-67. Analyses of these factors were beyond the scope of the current research and their use in prognosis has been summarised elsewhere (Blackwood et al., 2012; Kiupel & Camus, 2019).

This research was restricted to the data available from the retrospective review of patient clinical records from the participating primary veterinary clinics. Consequently, the data were limited regarding the cytological or histological parameters used for the detection and confirmation of tumour metastasis at the time of excision and this was further affected by interpreter bias between clinicians and between veterinary practices. Further, the de novo or recurrent/metastatic origin of secondary lesions could not be determined from the recorded data. A prospective study design would have allowed more accurate data collection.

5 | CONCLUSIONS

The relationships between prognostic, clinical, and survival data for cutaneous or subcutaneous MCTs in dogs presented to first opinion veterinary practices in south and south-eastern Australia were explored. After including tumour type in the multivariable models, dogs with *KIT* exon 11 ITD-mutant MCTs were not at an increased risk of MCT-related death or second MCT diagnosis. The diagnosis of a cutaneous Kiupel high-grade MCT was a statistically significant risk factor for 6- and 12-month MCT-related death and second MCT diagnosis, highlighting histological grade as the single most important prognostic factor for dogs with cutaneous MCT. Still, it should be emphasised that no single clinical parameter should be used to define the prognosis for any given patient. Simultaneous assessment of clinical, pathological, and molecular parameters is likely to provide the most informed decision concerning patient prognosis and tumour therapeutic management.

An additional finding inferred from this work surrounds the predisposition of senior Australian Labrador Retriever dogs for developing high-grade MCTs. This tendency may suggest an underlying genetic predisposing element within this breed.

ACKNOWLEDGEMENT

The authors are grateful to Gribbles Veterinary Pathology for supplying the FFPE MCT blocks and to Dr Michelle Hebart for her statistical assistance.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICS STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. Approval (S2016-131) was received from the University of Adelaide Animal Ethics Committee. All data were collected in compliance with the ethical statements from Gribbles Veterinary Pathology.

AUTHOR CONTRIBUTIONS

Conceptualisation, data curation, formal analysis, methodology, investigation, writing original draft and review and editing, and visualisation: Vanessa Tamlin. *Supervision, methodology, resources, and writing (review and editing)*: Cynthia Bottema. *Resources, investigation, and writing (review and editing)*: Lucy Woolford. *Resources, investigation, and writing (review and editing)*: Elizabeth Dobson. *Mentorship and supervision, resources, investigation, and writing (review and editing)*: Allan Kessell. *Conceptualisation, funding acquisition, investigation, resources, mentorship and supervision, project administration, and writing (review and editing)*: Anne Peaston.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1002/vms3.812.

ORCID

Vanessa S. Tamlin https://orcid.org/0000-0003-2732-5369
Cynthia D. K. Bottema https://orcid.org/0000-0001-6245-0099
Lucy Woolford https://orcid.org/0000-0001-7271-2937
Anne E. Peaston https://orcid.org/0000-0002-7501-2415

REFERENCES

Arendt, M. L., Melin, M., Tonomura, N., Koltookian, M., Courtay-Cahen, C., Flindall, N., Bass, J., Boerkamp, K., Megquir, K., Youell, L., Murphy, S., Mccarthy, C., London, C., Rutteman, G. R., Starkey, M., & Lindblad-Toh, K. (2015). Genome-wide association study of golden retrievers identifies germ-line risk factors predisposing to mast cell tumours. *PLoS Genetics*, *11*(11), e1005647. https://doi.org/10.1371/journal.pgen.1005647

Baginski, H., Davis, G., & Bastian, R. P. (2014). The prognostic value of lymph node metastasis with grade 2 MCTs in dogs: 55 cases (2001-2010). *Journal of the American Animal Hospital Association*, *50*(2), 89–95. https://doi.org/10.5326/JAAHA-MS-5997

Beasley, T. M., & Schumacker, R. E. (1995). Multiple regression approach to analyzing contingency tables: Post hoc and planned comparison procedures. *The Journal of Experimental Education*, *64*(1), 79–93. https://doi.org/10.1080/00220973.1995.9943797

Blackwood, L., Murphy, S., Buracco, P., De Vos, J. P., De Fornel-Thibaud, P., Hirschberger, J., Kessler, M., Pastor, J., Ponce, F., Savary-Bataille, K., & Argyle, D. J. (2012). European consensus document on mast cell tumours in dogs and cats. *Veterinary and Comparative Oncology*, *10*(3), e1–e29. https://doi.org/10.1111/j.1476-5829.2012.00341.x

Bursac, Z., Gauss, C. H., Williams, D. K., & Hosmer, D. W. (2008). Purposeful selection of variables in logistic regression. *Source Code for Biology and Medicine*, *3*(1), 17. https://doi.org/10.1186/1751-0473-3-17

Downing, S., Chien, M. B., Kass, P. H., Moore, P. E., & London, C. A. (2002). 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*, *63*(12), 1718–1723. https://doi.org/10.2460/ajvr.2002.63.1718

Furitsu, T., Tsujimura, T., Tono, T., Ikeda, H., Kitayama, H., Koshimizu, U., Sugahara, H., Butterfield, J. H., Ashman, L. K., & Kanayama, Y. (1993). Identification of mutations in the coding sequence of the proto-oncogene c-kit in a human mast cell leukemia cell line causing ligand-independent activation of c-kit product. *Journal of Clinical Investigation*, *92*(4), 1736–1744. https://doi.org/10.1172/JCI116761

García-Pérez, M. A., & Núñez-Antón, V. (2016). Cellwise residual analysis in two-way contingency tables. *Educational and Psychological Measurement*, *63*(5), 825–839. https://doi.org/10.1177/0013164403251280

Giantin, M., Vascellari, M., Morello, E. M., Capello, K., Vercelli, A., Granato, A., Lopparelli, R. M., Nassuato, C., Carminato, A., Martano, M., Mutinelli, F., & Dacasto, M. (2012). c-KIT messenger RNA and protein expression and mutations in canine cutaneous mast cell tumors: Correlations with post-surgical prognosis. *Journal of Veterinary Diagnostic Investigation*, *24*(1), 116–126. https://doi.org/10.1177/1040638711425945

Greer, K. A., Canterberry, S. C., & Murphy, K. E. (2007). Statistical analysis regarding the effects of height and weight on life span of the domestic dog. *Research in Veterinary Science*, *82*(2), 208–214. https://doi.org/10.1016/j.rvsc.2006.06.005

Horta, R. S., Lavalle, G. E., Monteiro, L. N., Souza, M. C. C., Cassali, G. D., & Araujo, R. B. (2018). Assessment of canine mast cell tumor mortality risk based on clinical, histologic, immunohistochemical, and molecular features. *Veterinary Pathology*, *55*(2), 212–223. https://doi.org/10.1177/0300985817747325

Jaensch, S. M. (2008). Canine mast cell neoplasia in Australia: A retrospective study of 909 cases (2006-2007). *Australian Veterinary Practitioner*, *38*(3), 115–119.

Kiupel, M. 2016. Mast cell tumors. InD. J. Meuten (Ed.), *Tumors in domestic animals* (5th ed., pp. 176–202). John Wiley & Sons.

Kiupel, M., & Camus, M. (2019). Diagnosis and prognosis of canine cutaneous mast cell tumors. *Veterinary Clinics of North America: Small Animal Practice*, *49*(5), 819–836. https://doi.org/10.1016/j.cvsm.2019.04.002

Kiupel, M., Webster, J. D., Bailey, K. L., Best, S., Delay, J., Detrisac, C. J., Fitzgerald, S. D., Gamble, D., Ginn, P. E., Goldschmidt, M. H., Hendrick, M. J., Howerth, E. W., Janovitz, E. B., Langohr, I., Lenz, S. D., Lipscomb, T. P., Miller, M. A., Misdorp, W., Moroff, S., … Miller, R. (2011). Proposal of a 2-tier histologic grading system for canine cutaneous mast cell tumors to more accurately predict biological behavior. *Veterinary Pathology*, *48*(1), 147–155. https://doi.org/10.1177/0300985810386469

Kiupel, M., Webster, J. D., Miller, R. A., & Kaneene, J. B. (2005). Impact of tumour depth, tumour location and multiple synchronous masses on the prognosis of canine cutaneous mast cell tumours. *Journal of Veterinary Medicine. A: Physiology, Pathology, Clinical Medicine*, *52*(6), 280–286. https://doi.org/10.1111/j.1439-0442.2005.00726.x

Leidinger, E. F., Freeman, K., Kirtz, G., Hooijberg, E. H., & Sick, K. (2014). Breed related odds ratio and anatomic distribution of canine mast cell tumours in Austria. Retrospective study of cases in the years 2000–2010. *Tierarztl Prax Ausg K Kleintiere Heimtiere*, *42*(6), 367–373. doi:10.15654/TPK-140165

Lennartsson, J., Jelacic, T., Linnekin, D., & Shivakrupa, R. (2005). Normal and oncogenic forms of the receptor tyrosine kinase kit. *Stem Cells*, *23*(1), 16–43. https://doi.org/10.1634/stemcells.2004-0117

Letard, S., Yang, Y., Hanssens, K., Palmerini, F., Leventhal, P. S., Guery, S., Moussy, A., Kinet, J. P., Hermine, O., & Dubreuil, P. (2008). Gain-of-function mutations in the extracellular domain of KIT are common in canine mast cell tumors. *Molecular Cancer Research*, *6*(7), 1137–1145. https://doi.org/10.1158/1541-7786.MCR-08-0067

London, C. A., Galli, S. J., Yuuki, T., Hu, Z. Q., Helfand, S. C., & Geissler, E. N. (1999). Spontaneous canine mast cell tumors express tandem duplications in the proto-oncogene c-kit. *Experimental Hematology*, *27*(4), 689–697. https://doi.org/10.1016/s0301-472x(98)00075-7

Marconato, L., Polton, G., Stefanello, D., Morello, E., Ferrari, R., Henriques, J., Tortorella, G., Benali, S. L., Bergottini, R., Vasconi, M. E., Annoni, M., & Sabattini, S. (2018). Therapeutic impact of regional lymphadenectomy in canine stage II cutaneous mast cell tumours. *Veterinary and Comparative Oncology*, *16*(4), 580–589. https://doi.org/10.1111/vco.12425

Mochizuki, H., Motsinger-Reif, A., Bettini, C., Moroff, S., & Breen, M. (2017). Association of breed and histopathological grade in canine mast cell tumours. *Veterinary and Comparative Oncology*, *15*(3), 829–839. https://doi.org/10.1111/vco.12225

Mullins, M. N., Dernell, W. S., Withrow, S. J., Ehrhart, E. J., Thamm, D. H., & Lana, S. E. (2006). Evaluation of prognostic factors associated with outcome in dogs with multiple cutaneous mast cell tumors treated with surgery with and without adjuvant treatment: 54 cases (1998-2004). *Journal of the American Veterinary Medical Association*, *228*(1), 91–95. https://doi.org/10.2460/javma.228.1.91

Murphy, S., Sparkes, A. H., Blunden, A. S., Brearley, M. J., & Smith, K. C. (2006). Effects of stage and number of tumours on prognosis of dogs with cutaneous mast cell tumours. *Veterinary Record*, *158*(9), 287–291. https://doi.org/10.1136/vr.158.9.287

Murphy, S., Sparkes, A. H., Smith, K. C., Blunden, A. S., & Brearley, M. J. (2004). Relationships between the histological grade of cutaneous mast cell tumours in dogs, their survival and the efficacy of surgical resection. *Veterinary Record*, *154*(24), 743–746. https://doi.org/10.1136/vr.154.24.743

Nakano, Y., Kobayashi, M., Bonkobara, M., & Takanosu, M. (2017). Identification of a secondary mutation in the KIT kinase domain correlated with imatinib-resistance in a canine mast cell tumor. *Veterinary Immunology and Immunopathology*, *188*, 84–88. https://doi.org/10.1016/j.vetimm.2017.05.004

Newman, S. J., Mrkonjich, L., Walker, K. K., & Rohrbach, B. W. (2007). Canine subcutaneous mast cell tumour: Diagnosis and prognosis. *Journal of Comparative Pathology*, *136*(4), 231–239. https://doi.org/10.1016/j.jcpa.2007.02.003

O'connell, K., & Thomson, M. (2013). Evaluation of prognostic indicators in dogs with multiple, simultaneously occurring cutaneous mast cell tumours: 63 cases. *Veterinary and Comparative Oncology*, *11*(1), 51–62. https://doi.org/10.1111/j.1476-5829.2011.00301.x

Patnaik, A. K., Ehler, W. J., & Macewen, E. G. (1984). Canine cutaneous mast cell tumor: Morphologic grading and survival time in 83 dogs. *Veterinary Pathology*, *21*(5), 469–474. https://doi.org/10.1177/030098588402100503

Pierini, A., Lubas, G., Gori, E., Binanti, D., Millanta, F., & Marchetti, V. (2019). Epidemiology of breed-related mast cell tumour occurrence and prognostic significance of clinical features in a defined population of dogs in west-central Italy. *Veterinary Science*, *6*(2), 53. https://doi.org/10.3390/vetsci6020053

Reguera, M. J., Ferrer, L., & Rabanal, R. M. (2002). Evaluation of an intron deletion in the c-kit gene of canine mast cell tumors. *American Journal of Veterinary Research*, *63*(9), 1257–1261. https://doi.org/10.2460/ajvr.2002.63.1257

Reynolds, B. D., Thomson, M. J., O'connell, K., Morgan, E. J., & Gummow, B. (2019). Patient and tumour factors influencing canine mast cell tumour histological grade and mitotic index. *Veterinary and Comparative Oncology*, *17*(3), 338–344. https://doi.org/10.1111/vco.12477

Riva, F., Brizzola, S., Stefanello, D., Crema, S., & Turin, L. (2005). A study of mutations in the c-kit gene of 32 dogs with mastocytoma. *Journal of Veterinary Diagnostic Investigation*, *17*(4), 385–388. https://doi.org/10.1177/104063870501700416

Smiech, A., Lopuszynski, W., Slaska, B., Bulak, K., & Jasik, A. (2019). Occurrence and distribution of canine cutaneous mast cell tumour characteristics among predisposed breeds. *Journal of Veterinary Research*, *63*(1), 141–148. https://doi.org/10.2478/jvetres-2019-0002

Smiech, A., Slaska, B., Lopuszynski, W., Jasik, A., Bochynska, D., & Dabrowski, R. (2018). Epidemiological assessment of the risk of canine mast cell tumours based on the Kiupel two-grade malignancy classification. *Acta Veterinaria Scandinavica*, *60*(1), 70. https://doi.org/10.1186/s13028-018-0424-2

Stefanello, D., Buracco, P., Sabattini, S., Finotello, R., Giudice, C., Grieco, V., Iussich, S., Tursi, M., Scase, T., Di Palma, S., Bettini, G., Ferrari, R., Martano, M., Gattino, F., Marrington, M., Mazzola, M., Elisabetta Vasconi, M., Annoni, M., & Marconato, L. (2015). 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*, *246*(7), 765–769. https://doi.org/10.2460/javma.246.7.765

Tamlin, V. S., Bottema, C. D. K., & Peaston, A. E. (2020). Comparative aspects of mast cell neoplasia in animals and the role of KIT in prognosis and treatment. *Veterinary Medicine and Science*, *6*(1), 3–18.

Tamlin, V. S., Dobson, E. C., Woolford, L., & Peaston, A. E. (2019). DNA purification increases PCR-amplifiable DNA extracted from formalin-fixed, paraffin-embedded canine mast cell tumors for routine KIT mutation detection. *Journal of Veterinary Diagnostic Investigation*, *31*(5), 756–760. https://doi.org/10.1177/1040638719867743

Tamlin, V. S., Kessell, A. E., Mccoy, R. J., Dobson, E. C., Smith, T. S., Hebart, M., Brown, L., Mitrovic, D., & Peaston, A. E. (2017). Prevalence of exon 11 internal tandem duplications in the C-KIT proto-oncogene in Australian canine mast cell tumours. *Australian Veterinary Journal*, *95*(10), 386–391. https://doi.org/10.1111/avj.12636

Thamm, D. H., Avery, A. C., Berlato, D., Bulman-Fleming, J., Clifford, C. A., Hershey, A. E., Intile, J. L., Jones, P. D., Kamstock, D. A., Liptak, J. M., Pavuk, A., Peauroi, J., Powell, R., Rissetto, K., Valli, V. E. O., & Webster, J. D. (2019). Prognostic and predictive significance of KIT protein expression

and c-kit gene mutation in canine cutaneous mast cell tumours: A consensus of the Oncology-Pathology Working Group. *Veterinary and Comparative Oncology*, *17*(4), 451–455. https://doi.org/10.1111/vco.12518

Thompson, J. J., Pearl, D. L., Yager, J. A., Best, S. J., Coomber, B. L., & Foster, R. A. (2011). Canine subcutaneous mast cell tumor: characterization and prognostic indices. *Veterinary Pathology*, *48*(1), 156–168. https://doi.org/10.1177/0300985810387446

Thompson, J. J., Yager, J. A., Best, S. J., Pearl, D. L., Coomber, B. L., Torres, R. N., Kiupel, M., & Foster, R. A. (2011). Canine subcutaneous mast cell tumors: cellular proliferation and KIT expression as prognostic indices. *Veterinary Pathology*, *48*(1), 169–181. https://doi.org/10.1177/0300985810390716

Vozdova, M., Kubickova, S., Fictum, P., Cernohorska, H., Frohlich, J., & Rubes, J. (2020). Mutation and methylation status of KIT and TP53 in canine cutaneous and subcutaneous mast cell tumours. *Veterinary and Comparative Oncology*, *18*(3), 438–444. https://doi.org/10.1111/vco.12543

Warland, J., & Dobson, J. (2013). Breed predispositions in canine mast cell tumour: A single centre experience in the United Kingdom. *Veterinary Journal*, *197*(2), 496–498. https://doi.org/10.1016/j.tvjl.2013.02.017

Webster, J. D., Yuzbasiyan-Gurkan, V., Kaneene, J. B., Miller, R., Resau, J. H., & Kiupel, M. (2006). The role of c-KIT in tumorigenesis: Evaluation in canine cutaneous mast cell tumors. *Neoplasia*, *8*(2), 104–111. https://doi.org/10.1593/neo.05622

Webster, J. D., Yuzbasiyan-Gurkan, V., Thamm, D. H., Hamilton, E., & Kiupel, M. (2008). Evaluation of prognostic markers for canine mast cell tumors treated with vinblastine and prednisone. *BMC Veterinary Research*, *4*(1), 32. https://doi.org/10.1186/1746-6148-4-32

Weishaar, K. M., Thamm, D. H., Worley, D. R., & Kamstock, D. A. (2014). 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*, *151*(4), 329–338. https://doi.org/10.1016/j.jcpa.2014.07.004

White, C. R., Hohenhaus, A. E., Kelsey, J., & Procter-Gray, E. (2011). Cutaneous MCTs: Associations with spay/neuter status, breed, body size, and phylogenetic cluster. *Journal of the American Animal Hospital Association*, *47*(3), 210–216. https://doi.org/10.5326/JAAHA-MS-5621

How to cite this article: Tamlin, V. S, Bottema, C. D. K, Woolford, L., Dobson, E. C, Kessell, A. E, & Peaston, A. E. (2022). Canine mast cell tumours part I: Clinical and survival outcomes. *Veterinary Medicine and Science*, *8*, 1409–1420. https://doi.org/10.1002/vms3.812