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REVIEW ARTICLE

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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

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Abstract

One of the primary objectives of the Oncology-Pathology Working Group (OPWG), a joint initiative of the Veterinary Cancer Society and the American College of Veterinary Pathologists, is for oncologists and pathologists to collaboratively generate consensus documents to standardize aspects of and provide guidelines for oncologic pathology. Consensus is established through critical review of peer-reviewed literature relevant to a subgroup's particular focus. Subsequent acceptance and approval of the document by the OPWG membership at large establishes consensus. The intent of this publication is to help educate practitioners and pathologists on the value of diagnostics related to the KIT receptor tyrosine kinase for canine cutaneous mast cell tumours and to provide a guide for the use of these tests in veterinary medicine. This document represents the opinions of the OPWG and the authors and does not constitute a formal endorsement by the American College of Veterinary Pathologists or the Veterinary Cancer Society.

KEYWORDS

cancer, dog, immunohistochemistry, kinase, mutation

1 | INTRODUCTION

Mast cell tumours (MCT) are the most common malignant cutaneous tumours in the dog, accounting for between 16 and 21% of all cutaneous neoplasms.[1,2] While the majority of canine MCT can be effectively treated with local therapy (surgery ± radiation therapy), a subset of tumours can be associated with a high risk of metastasis and accordingly short overall survival times. Furthermore, locally recurrent, large or infiltrative tumours, and those in locations not amenable to wide surgical excision represent a therapeutic challenge. Histologic appearance (eg, grade, mitotic activity) remains one of the mainstays for determining likely biologic behavior[3,4]; however, a subset of MCT may behave aggressively despite an unremarkable histologic appearance. Additional tests, to better predict potential biological behaviour and clinical outcome and identify patients that may benefit from adjuvant medical therapy, would be useful. Furthermore, given the recent expansion of medical options for canine MCT treatment, including a variety of cytotoxic agents as well as small-molecule tyrosine kinase inhibitors (TKIs), information that could aid in predicting drug response, and aid in the selection of effective treatment, would be likewise helpful.

Mutations in the *c-kit* gene have been identified in approximately 15% of canine cutaneous MCT, with an increased incidence up to 35% in higher grade MCT. Internal tandem duplications (ITD) in exon 11 are the most commonly characterized *c-kit* gene mutations in MCT,[5-8] but lower numbers of deletion mutations have been identified in exon 11, and ITDs and substitutions have been identified in exons 8 and 9.[5] Rare mutations have also been identified in exon 17.[5] To date, all mutations that have been characterized *in vitro* have been shown to result in constitutive autophosphorylation of the KIT protein in the absence of its ligand, stem cell factor (SCF).[5] Additionally, TKIs targeting KIT have been shown to inhibit phosphorylation of these mutated proteins, except for those resulting from exon 17 mutations.[5] Furthermore, studies have demonstrated that a proportion of canine MCT may demonstrate aberrant subcellular localization of the KIT protein when assessed via immunohistochemistry (IHC), and that this aberrant localization may correlate with the presence of *c-kit* gene mutation as well as outcome.[9]

Multiple studies have sought to evaluate the significance of the presence of activating *c-kit* mutations and/or aberrant KIT protein localization on postsurgical outcome in dogs with MCT.[9-16] Furthermore, some studies have begun to evaluate whether these factors may affect outcome with medical therapy, specifically with TKIs.[17-24] The goal of this review is to summarize the available data regarding the utility of *c-kit* gene mutation and KIT protein localization as prognostic and predictive tests for canine MCT.

1.1 | Prognostic value of c-kit gene mutation

Most studies evaluating the association between *c-kit* gene mutations and prognosis have been focused on ITD mutations in exon 11. Several studies have found increased ITDs and deletions in higher histologic grade MCT.[15,25,26] In one study, MCT with ITD mutations were twice as likely to recur or develop metastases. These results were not statistically significant, but might be influenced by the relatively small numbers of low-grade tumours included in this study.[15] Additionally, Webster et al found that dogs whose MCT possessed ITD mutations had significantly decreased overall survival times and an increased incidence of MCT-related death and recurrence when treated with surgery, and/or when treated with multimodality therapy.[10,27] Other studies have found statistical associations between exon 11 mutations and increased cellular proliferation indices (mitotic count, AgNOR frequency, Ki67 index).[26,28] In a prospective study evaluating the TKI masitinib in dogs with measurable MCT, patients in the placebo arm of this study with *c-kit* gene mutations experienced shorter times to progression and overall survival times compared with patients without mutations (42 vs 98 and 182 days vs median not reached, respectively), although this was not evaluated statistically.[17] However, in a study by Giantin et al,[13] only one of five patients with *c-kit* mutations had recurrent disease and MCT-related mortality.

Given its documented strong association with both histologic grade and proliferation indices,[15,25,26,28] it is not clear whether the presence of a *c-kit* gene mutation represents an independent prognostic factor, or whether it may largely correlate with histologic grade/proliferation rate, which can be assessed more simply and inexpensively. However, mutation presence/absence is a more objective measurement than either histologic grade or mitotic index/count, both of which can be associated with considerable observer bias. Thus, this measure could still provide important objective information useful in decision-making.

This body of data suggests that patients with *c-kit* gene mutations are more likely to have aggressive disease as measured by increased recurrence rates and decreased survival times however, the presence of a *c-kit* mutation has not been definitively validated as an independent prognostic factor, when taking into account known factors such as histologic grade and proliferation rate. One recent study found that the presence of *c-kit* mutations was predictive of outcome on univariate, but not multivariate analysis.[26]

1.2 | Prognostic value of KIT protein localization

Reguera et al[29] first described variations in KIT expression in canine MCT by IHC. In this study, it was noted that Patnaik grade I MCTs had weak KIT labelling scattered in the cytoplasm or on the membrane, while grade II and III tumours tended to have increased cytoplasmic labelling. Evaluations of KIT localization and prognosis have produced variable results. In three studies, patients with focal or diffuse cytoplasmic KIT expression had a worse postsurgical prognosis, either in terms of recurrence and/or survival, compared with MCTs with peri-membrane labeling.[12,13,26,27] Although cytoplasmic KIT localization was associated with a worse prognosis in these studies, it had low positive predictive value, suggesting that membrane localization was fairly predictive of a good prognosis, but cytoplasmic KIT expression could not clearly delineate aggressive disease.

In contrast to the studies described above, Costa Casagrande et al[14] found no association with KIT staining pattern and histologic grade or survival measures, and Preziosi et al[30] found that focal perinuclear labelling was associated with a worse prognosis than diffuse cytoplasmic labelling, but too few patients with membranous labelling were evaluated to comment on associations with survival.[30] A technical concern with the varying results of these studies is that the IHC labelling procedure can influence the interpretation of KIT protein localization. Specifically, over-developing the IHC reaction can result in increased cytoplasmic background in MCTs, so it appears as weak, diffuse cytoplasmic labelling. This is especially noteworthy in Preziosi study where the image of diffuse cytoplasmic labelling demonstrates strong membrane labelling with weaker cytoplasmic labelling. Therefore, some membrane localizing tumours may be misclassified as diffuse cytoplasmic labelling. A potential way to control for this would be to include a tissue section with normal mast cells. These cells should have KIT restricted to the plasma membrane and therefore would serve as a positive control that the reaction was performed appropriately.[31]

As above regarding *c-kit* gene mutation, given the potential correlation between KIT protein localization and other validated prognostic factors (grade, proliferation),[13,26,28,29] it is similarly unclear whether KIT localization represents an independent prognostic factor, when taking into account these other features. In one comparatively large retrospective study, KIT localization was significant on univariate analysis but lost prognostic value upon multivariate analysis[26]; however, in another prospective study evaluating medical therapy for measurable MCT, KIT localization did retain independent prognostic value upon multivariate analysis.[18]

This body of literature suggests that increased cytoplasmic KIT localization is associated with worse prognosis as measured by recurrence and survival, but it has a low positive predictive value and therefore should not be used alone. More power likely lies in using membrane localization to rule out potentially aggressive tumours.

1.3 | Value of c-kit mutation status and KIT localization in predicting response to therapy

The potential predictive value of *c-kit* mutation status in predicting outcome following treatment with the TKIs toceranib phosphate and masitinib has been evaluated to some degree in the two published registration trials for these respective agents. In the toceranib registration study as well as in preliminary investigations, patients with *c-kit* gene mutations had objective response rates twice as high as those without mutations (60% vs 30%), although effect on long-term outcome (progression free interval, overall survival) was not reported.[19,20] In the masitinib registration study, a significant difference in outcome between masitinib and placebo arms was observed only in the patients with *c-kit* mutations. Additionally, patients in the masitinib arm of this study with *c-kit* mutations appeared to have longer times to progression (230 vs 83 days) and maintained higher overall response rates at 6 months (20% vs 10%), although these differences were not evaluated statistically.[17] A similar observation was made in a small number of dogs treated with the KIT TKI imatinib; dogs with *c-kit* exon 11 ITDs were numerically more likely to experience objective responses to imatinib, although long-term outcomes were not reported.[21] However, some recent studies have suggested no correlation between TKI response and *c-kit* mutational status.[18,22]

Interestingly, patients with *c-kit* mutations had significantly decreased progression free survival times compared with those without *c-kit* mutations in a single arm study of toceranib and hypofractionated radiation therapy, although this was evaluated in a small number of patients,[24] and a recent comparatively large multicentre prospective study suggested a similar negative correlation between *c-kit* mutation status and outcome following toceranib treatment.[18] These results are important as they suggest that, even if initial response rate may be increased in dogs whose MCT possess *c-kit* mutations, this may not translate into improvements in long-term outcome.

It is noteworthy that patient subsets without *c-kit* mutations have been demonstrated to respond to TKI therapy.[17,19,20] This may be due to non-mutational activation of KIT (eg, autocrine or paracrine signalling, amplifications), the presence of activating mutations not screened for as part of testing, or inhibition of other tyrosine kinases (eg, PDGFR, VEGFR2), as these drugs are not 100% selective for KIT.

The effects of KIT localization on outcome following TKI treatment have been assessed in two recent studies.[18,22] Neither study detected a correlation between KIT localization and objective response (although one was relatively small and thus underpowered), but in the larger, prospective study, aberrant KIT localization was associated with inferior progression free and overall survival time following toceranib treatment.[18]

2 | CONCLUSIONS

In summary, no prognostic marker can be considered to have 100% positive and negative predictive values. Instead, all prognostic markers can provide varying levels of risk assessment or hazard ratios. Comorbidities, disease heterogeneity, multigenic influences, variations in tolerance of adverse effects and ability to treat will all impact the clinical course of a given patient. Therefore, the greatest prognostic benefit will likely stem from using multiple prognostic markers in concert. *c-kit* gene mutation analysis and KIT localization may be most be informative in histologically "ambiguous" tumours. *c-kit* gene mutations may be most informative in the identification of tumours that are histologically low grade, but are likely to be biologically aggressive, while membrane KIT localization is most likely to identify tumours that are less likely to have progressive disease. The available literature is mixed, but *c-kit* mutation assessment may have some ability to determine which patients are likely to experience an initial response to single agent TKIs. This could be especially useful in those cases where "neoadjuvant" medical cytoreduction may facilitate surgical excision, although this may not translate into long-term clinical benefit. It is worth noting that conflicting results may be encountered (eg, a high-grade tumour with membranous KIT localization, a low-grade

tumour with a *c-kit* mutation). In these situations, clinical judgement and/or additional testing must be integrated into the decision process.

2.1 | Future directions/additional studies

Multiple unanswered questions remain. What is the incidence of *c-kit* gene mutation and/or aberrant KIT protein localization specifically in "low risk" (eg, low-mitotic Patnaik grade I/II, 2-tier low-grade) tumours, and does the presence of a *c-kit* mutation and/or aberrant KIT localization have an effect on outcome in these "low risk" patients? Are *c-kit* mutations and KIT localization independent prognostic factors when taking into account known factors such as histologic grade and proliferation index? Do these same factors carry prognostic significance in patients treated with chemotherapy or radiation therapy?

Further investigations should be conducted to better determine if there are differences in the prognostic significance of mutations located in various exons, since most work to date has focused on exon 11.

The predictive value of KIT IHC in assessing masitinib response remains to be evaluated, which would be best in a prospective study.

Additionally, a multicentre prospective study to evaluate histologic grade, mitotic index, proliferation markers, *c-kit* mutations and KIT localization in concert on postsurgical outcome is greatly needed; not only to determine how these can be used in concert, but also to standardize criteria for these assessments. The OPWG would be an optimal forum to facilitate organization such a multicentre study, since it will require integration of pathologists, oncologists, surgeons and molecular biologists.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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REFERENCES

*These references are cited, as appropriate; however, they were not part of the critically reviewed literature by the subgroup in establishing consensus.

1. Bostock DE. Neoplasms of the skin and subcutaneous tissues in dogs and cats. *Br Vet J*. 1986;142(1):1-19.*
2. Brodey RS. Canine and feline neoplasia. *Adv Vet Sci Comp Med*. 1970; 14:309-354.*
3. Patnaik AK, Ehler WJ, MacEwen EG. Canine cutaneous mast cell tumor: morphologic grading and survival time in 83 dogs. *Vet Pathol*. 1984;21(5):469-474.*
4. Kiupel M, Webster JD, Bailey KL, et al. Proposal of a 2-tier histologic grading system for canine cutaneous mast cell tumors to more accurately predict biological behavior. *Vet Pathol*. 2011;48(1):147-155.*
5. Letard S, Yang Y, Hanssens K, et al. Gain-of-function mutations in the extracellular domain of KIT are common in canine mast cell tumors. *Mol Cancer Res*. 2008;6(7):1137-1145.
6. London CA, Galli SJ, Yuuki T, Hu ZQ, Helfand SC, Geissler EN. Spontaneous canine mast cell tumors express tandem duplications in the proto-oncogene c-kit. *Exp Hematol*. 1999;27(4):689-697.
7. Ma Y, Longley BJ, Wang X, Blount JL, Langley K, Caughey GH. Clustering of activating mutations in c-KIT's juxtamembrane coding region of canine mast cell neoplasms. *J Invest Dermatol*. 1999;112:165-170.
8. Riva F, Brizzola S, Stefanello D, Crema S, Turin L. A study of mutations in the c-kit gene of 32 dogs with mastocytoma. *J Vet Diagn Investig*. 2005;17(4):385-388.
9. Webster JD, Kiupel M, Yuzbasiyan-Gurkan V. Evaluation of the kinase domain of c-KIT in canine cutaneous mast cell tumors. *BMC Cancer*. 2006;6:85.
10. Webster JD, Yuzbasiyan-Gurkan V, Thamm DH, Hamilton E, Kiupel M. Evaluation of prognostic markers for canine mast cell tumors treated with vinblastine and prednisone. *BMC Vet Res*. 2008; 4:32.
11. Thompson JJ, Morrison JA, Pearl DL, et al. Receptor tyrosine kinase expression profiles in canine cutaneous and subcutaneous mast cell tumors. *Vet Pathol*. 2016;53(3):545-558.
12. Thompson JJ, Yager JA, Best SJ, et al. Canine subcutaneous mast cell tumors: cellular proliferation and KIT expression as prognostic indices. *Vet Pathol*. 2011;48(1):169-181.
13. Giantin M, Vascellari M, Morello EM, et al. c-KIT messenger RNA and protein expression and mutations in canine cutaneous mast cell tumors: correlations with post-surgical prognosis. *J Vet Diagn Investig*. 2012;24(1):116-126.
14. Costa Casagrande TA, de Oliveira Barros LM, Fukumasu H, et al. The value of molecular expression of KIT and KIT ligand analysed using real-time polymerase chain reaction and immunohistochemistry as a prognostic indicator for canine cutaneous mast cell tumours. *Vet Comp Oncol*. 2015;13(1):1-10.
15. Downing S, Chien MB, Kass PH, Moore PE, London CA. Prevalence and importance of internal tandem duplications in exons 11 and 12 of c-kit in mast cell tumors of dogs. *Am J Vet Res*. 2002;63(12):1718-1723.
16. Webster JD, Kiupel M, Kaneene JB, Miller R, Yuzbasiyan-Gurkan V. The use of KIT and tryptase expression patterns as prognostic tools for canine cutaneous mast cell tumors. *Vet Pathol*. 2004;41(4): 371-377.
17. Hahn KA, Ogilvie G, Rusk T, et al. Masitinib is safe and effective for the treatment of canine mast cell tumors. *J Vet Intern Med*. 2008;22 (6):1301-1309.
18. Weishaar KM, Ehrhart EJ, Avery AC, et al. c-Kit mutation and localization status as response predictors in mast cell tumors in dogs treated with prednisone and toceranib or vinblastine. *J Vet Intern Med*. 2018; 32(1):394-405.
19. London CA, Malpas PB, Wood-Follis SL, et al. Multi-center, placebo-controlled, double-blind, randomized study of oral toceranib phosphate (SU11654), a receptor tyrosine kinase inhibitor, for the treatment of dogs with recurrent (either local or distant) mast cell tumor following surgical excision. *Clin Cancer Res*. 2009;15(11):3856-3865.
20. London CA, Hannah ALL, Zadovoskaya R, et al. Phase I dose-escalating study of SU11654, a small molecule receptor tyrosine kinase inhibitor, in dogs with spontaneous malignancies. *Clin Cancer Res*. 2003;9(7):2755-2768.

21. Isotani M, Ishida N, Tominaga M, et al. Effect of tyrosine kinase inhibition by imatinib mesylate on mast cell tumors in dogs. *J Vet Intern Med*. 2008;22(4):985-988.
22. Horta RDS, Giuliano A, Lavalle GE, et al. Clinical, histological, immunohistochemical and genetic factors associated with measurable response of high-risk canine mast cell tumours to tyrosine kinase inhibitors. *Oncol Lett*. 2018;15(1):129-136.
23. Burton JH, Venable RO, Vail DM, et al. Pulse-administered toceranib phosphate plus lomustine for treatment of unresectable mast cell tumors in dogs. *J Vet Intern Med*. 2015;29(4):1098-1104.
24. Carlsten KS, London CA, Haney S, Burnett R, Avery AC, Thamm DH. Multicenter prospective trial of hypofractionated radiation treatment, toceranib, and prednisone for measurable canine mast cell tumors. *J Vet Intern Med*. 2012;26(1):135-141.
25. Zemke D, Yamini B, Yuzbasiyan-Gurkan V. Mutations in the juxtamembrane domain of c-KIT are associated with higher grade mast cell tumors in dogs. *Vet Pathol*. 2002;39(5):529-535.
26. Horta RS, Lavalle GE, Monteiro LN, Souza MCC, Cassali GD, Araujo RB. Assessment of canine mast cell tumor mortality risk based on clinical, histologic, immunohistochemical, and molecular features. *Vet Pathol*. 2018;55(2):212-223.
27. Webster JD, Yuzbasiyan-Gurkan V, Kaneene JB, Miller R, Resau JH, Kiupel M. The role of c-KIT in tumorigenesis: evaluation in canine cutaneous mast cell tumors. *Neoplasia*. 2006;8(2):104-111.
28. Webster JD, Yuzbasiyan-Gurkan V, Miller RA, Kaneene JB, Kiupel M. Cellular proliferation in canine cutaneous mast cell tumors: associations with c-KIT and its role in prognostication. *Vet Pathol*. 2007;44(3):298-308.
29. Reguera MJ, Rabanal RM, Puigdemont A, Ferrer L. Canine mast cell tumors express stem cell factor receptor. *Am J Dermatopathol*. 2000;22(1):49-54.
30. Preziosi R, Sarli G, Paltrinieri M. Prognostic value of intratumoral vessel density in cutaneous mast cell tumors of the dog. *J Comp Pathol*. 2004;130(2–3):143-151.
31. Arber DA, Tamayo R, Weiss LM. Paraffin section detection of the c-kit gene product (CD117) in human tissues: value in the diagnosis of mast cell disorders. *Hum Pathol*. 1998;29(5):498-504.*

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