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Expression and prognostic significance of c-kit in canine mast cell tumors

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Abstract
Canine mast cell tumors are common cutaneous neoplasms with variable behavior. This study evaluated c-KIT marker expression in 30 cases to determine its diagnostic and prognostic significance through real time PCR and Immunohistochemistry. Immunohistochemistry identified three patterns: membranous (Pattern I), focal cytoplasmic (Pattern II), and diffuse cytoplasmic (Pattern III). Aberrant cytoplasmic localization (pattern II and III) was strongly associated with poor differentiation. Molecular analysis showed significant up regulation of c-KIT mRNA in grade III and high-grade tumors (Kruskal-Wallis, $p \leq 0.05$; Mann-Whitney, $p \leq 0.01$). Significant differences in mean c-KIT expression were also observed between recurrence and alive groups and between dead and alive groups. Kaplan-Meier analysis demonstrated markedly shorter survival (51.5 days) and earlier recurrence (110.1 days) in dogs with c-KIT overexpression, whereas survival was undefined for the under expression group. Overall, these findings underscore the diagnostic and prognostic relevance of c-KIT and support its use as a valuable marker in canine mast cell tumors.

Keywords: PCR, c-KIT, Canine mast cell tumor, immunohistochemistry, prognosis

Introduction
Canine mast cell tumors (MCTs) represent approximately 16-21% of all cutaneous neoplasms in dogs and display wide variability in biological behavior, ranging from benign, indolent forms to highly aggressive and metastatic variants (Welle *et al.*, 2008 and Blackwood *et al.*, 2012) [18, 2]. Accurate prognostication remains challenging due to this heterogeneity. Histopathological grading systems, such as those proposed by Patnaik *et al.* (1984) [12] and Kiupel *et al.* (2011) [9] serve as valuable diagnostic tools but have limitations in predicting recurrence, metastasis, and overall survival (Scase *et al.*, 2006 and Vascellari *et al.*, 2013) [13,16]. Consequently, identification of reliable molecular markers has gained importance in understanding tumor behavior and refining prognostic assessment.

The c-KIT proto-oncogene encodes a transmembrane receptor tyrosine kinase (RTK) that plays a crucial role in mast cell proliferation, differentiation, and survival (Besmer *et al.*, 1986; Yarden *et al.*, 1987 and Liao *et al.*, 2023) [1, 19, 10]. Activation occurs when stem cell factor (SCF) binds to the extracellular domain of the receptor, inducing dimerization and autophosphorylation of intracellular tyrosine residues. This event initiates downstream signaling cascades involving MAPK, PI3K, and Src family kinases, which regulate cellular growth and homeostasis. Mutations or overexpression of c-KIT can lead to ligand-independent activation, resulting in uncontrolled mast cell proliferation and neoplastic transformation (Hirota *et al.*, 1998 and Zemke *et al.*, 2002) [7, 20]. Gain-of-function mutations, particularly internal tandem duplications (ITDs) within exon 11, are the most frequently reported genetic alterations in canine MCTs and are associated with aggressive biological behavior, higher histologic grade, and poor prognosis (Webster *et al.*, 2006) [17]. Mutations in exons 8 and 9 have also been identified, with exon 8 alterations being more prevalent in subcutaneous MCTs, which tend to exhibit less aggressive clinical behavior (London *et al.*, 1999) [11]. Immunohistochemically, KIT expression patterns provide important prognostic insight.

Membranous localization (Pattern I) is typically associated with low-grade tumors, while focal (Pattern II) and diffuse cytoplasmic (Pattern III) expression patterns correlate with high-grade tumors, increased proliferation, and poorer outcomes (Kiupel *et al.*, 2004; Gil da Costa *et al.*, 2007; Thompson *et al.*, 2016 and de Nardi *et al.*, 2022) [8, 6, 15, 4]. Aberrant KIT expression and c-KIT mutations have been linked to decreased disease-free and overall survival (Webster *et al.*, 2006 and Tamlin *et al.*, 2020) [17,14]. Therefore, comprehensive evaluation of c-KIT expression and mutation status is essential in canine MCTs to refine prognosis, guide therapy, and establish standardized diagnostic protocols.

Materials and Methods

A prospective study was conducted over seven months at the Department of Veterinary Pathology, Veterinary College, Hebbal and Bengaluru. Out of 105 cutaneous tumor cases evaluated, 30 tumors were confirmed histopathologically as MCTs and molecular studies were conducted

Immunohistochemistry (IHC)

For c-KIT/CD117 detection, a polyclonal rabbit anti-human c-KIT antibody (Clone: A4502) was employed, procured from Dako, Agilent Technologies, USA. The same HRP goat anti-rabbit IgG (Clone: AS014) from Abclonal®, Woburn, USA served as the secondary antibody. The chromogenic detection utilized the DAB Substrate Kit from Thermo Scientific®, containing the same components as mentioned above. Sections were mounted on APES-coated slides, deparaffinized, and subjected to antigen retrieval in citrate buffer (10 mM, pH 6.0). C-KIT detection utilized rabbit polyclonal anti-human c-KIT antibody (1:100, Dako), HRP-conjugated secondary antibody, and DAB chromogen. Positive control included canine gastrointestinal stromal tumor (GIST) tissue. C-KIT expression patterns were classified as: Pattern I: Membranous; Pattern II: Focal cytoplasmic; Pattern III: Diffuse cytoplasmic localization.

Real-Time PCR (RT-PCR)

The representative tumor samples were collected in RNA later and stored at -80 °C. Total RNA was extracted by using TRIzol™ method. For complementary DNA synthesis, RevertAid™ First Strand cDNA Synthesis Kit (M/S Thermo Fisher Scientific) was used as per manufacturers recommendations. For this study, Primers for the target gene c-KIT was designed using the canine reference sequence available in the NCBI database (Accession No: AY313776.1) and housekeeping gene GAPDH was designed using the canine reference sequence available in the NCBI database (Accession No. BI817044.1), (Table.1)

(Tm conditions for c-KIT/ GAPGH in real time PCR: UDG activation 50 ºC for 2 minutes, initial denaturation 95 ºC for 5mins for 1 cycle followed by 35 cycles of Denaturation of cDNA at 95 ºC for 30 sec, annealing of primers at 57 ºC for 30 sec and extension at 70 ºC for 20 sec were carried out using CFX96 Real Time System, M/s BIO RAD CFX-96, USA). For relative quantification of gene transcription, the comparative Ct method was selected and values were expressed as relative to the reference sample (normal canine skin tissue), used as calibrator using 2-ΔΔCT method.

Statistical Analysis

To determine the prognostic value of c-KIT expression, the follow up details including post-surgical disease-free survival, recurrence, metastasis/ death were taken into the consideration for a period of 7 months. Further the values above and below the median expression value of c-KIT are considered as over and under expressed respectively. Data were analyzed with GraphPad Prism v8.4.3. Differences between tumor grades were assessed using Kruskal-Wallis and Mann-Whitney U tests. Survival outcomes were evaluated with Kaplan-Meier curves.

Ethical approval

The study protocol was reviewed and approved by Institutional Animal Ethics Committee (IAEC), Veterinary College, Hebbal, Bengaluru. All of the investigated samples were obtained for diagnostic purposes as part of routine and standard care. Procedures were designed to avoid or minimise discomfort, distress and pain. IAEC approval number: VCH/IAEC/2025/22.

Results

Real-time PCR (RT-PCR) was performed on the 30 tumor samples to quantify c-KIT mRNA expression, facilitating molecular correlation with immunohistochemical findings. The relative expression of c-KIT mRNA in 30 canine mast cell tumor (MCT) samples revealed a fold increase ranging from 7.49 to 92.73, with an overall mean (± SE) of 40.90±5.10. In contrast, normal skin tissue showed negligible c-KIT expression (Ct=30.54±1.02). Using GAPDH as the housekeeping gene, c-KIT expression levels showed a clear correlation with tumor grade. Based on Patnaik's classification, Grade III tumors exhibited the highest mean (±SE) expression (72.95±4.20), followed by Grade II (31.71±4.51) and Grade I (15.30±1.86), (Table 2). According to the Kiupel grading system, high-grade tumors demonstrated significantly higher c-KIT expression (59.43±5.61) compared to low-grade tumors (17.07±1.33) (Table 3). Statistical analysis confirmed a significant difference in c-KIT expression across tumor grades (Kruskal-Walli's test, $p \leq 0.05$; Mann-Whitney U test, $p \leq 0.01$), indicating progressive upregulation of c-KIT with increasing malignancy.

To evaluate the prognostic significance of c-KIT expression, cases were categorized based on the median fold-change value (28.01) into overexpression and underexpression groups (Table 4). Among 30 cases, recurrence occurred in 7 cases, death in 6 cases, and complete recovery in 17 cases. The mean (±SE) c-KIT expression values were markedly higher in the deceased (61.01±9.43) and recurrence (59.29±8.47) groups compared to the alive/disease-free group (27.55±5.46). Statistical comparison revealed significant differences between alive vs. recurrence and alive vs. death groups ($p \leq 0.05$), but not between recurrence and death groups ($p > 0.05$), (Table 5 and 5a). These results demonstrate that elevated c-KIT expression is closely associated with adverse clinical outcomes, including recurrence and mortality.

Kaplan-Meier survival analysis further supported these findings, showing a significant difference in survival duration between overexpression and underexpression groups (log-rank test, $p \leq 0.01$). Dogs with c-KIT overexpression had a median survival time of 51.5 days, whereas the underexpression group had an undefined median survival, indicating prolonged survival (Figure 1). Similarly, recurrence-free survival differed significantly between the groups ($p \leq 0.05$), with the overexpression group showing a median time to recurrence of 110.1 days (Figure 2.). In addition to RT-PCR, 30 canine mast cell tumor (MCT) tissue samples were subjected to immunohistochemistry, with

canine gastrointestinal stromal tumor serving as the positive control, exhibiting characteristic membranous and cytoplasmic brown staining. Three distinct c-KIT immunostaining patterns were identified: Pattern I (membranous) in 10 tumors, Pattern II (focal or stippled cytoplasmic) in 16 tumors, and Pattern III (diffuse cytoplasmic) in 4 tumors (Fig.03-06). Overall, these findings underscore c-KIT overexpression as a potential molecular marker of poor prognosis and shorter survival in canine mast cell tumors.

Table 1: RT-PCR primer details

Primer code	Sequence (5-3)	Primer length (bp)
c-KIT-F	ACCCAACACAGCTTCCTTAC	137 bp
c-KIT-R	GGCCGCATCCGACTTAAT	
GAPDH-F	GCTCCTCTAGCCAAAGTCATC	159 bp
GAPDH-R	GGAAGCAGGGATGATGTTCT	

Table 2: Kruskal Walli's test for comparison of mean (±SE) c-KIT values between various Patnaik grade mast cell tumors

Patnaik grade	No. of cases	Mean (±SE)
Grade I	9	15.30±1.86[a]
Grade II	13	31.71±4.51[b]
Grade III	8	72.95±4.20[c]
Significance		*

Note: Kruskal-Walli's test: *-Significant at $p \leq 0.05$: Mean (±SE) bearing different superscripts within a column are significantly different at $p \leq 0.05$.

Table 3: Mann Whitney test for comparison of mean (±SE) c-KIT values between High and low grade tumors

Kiupel grade	No of cases	Mean (±SE)
High	14	59.43±5.61[a]
Low	16	17.07±1.33[b]
Significance		**

Note: Mann Whitney test: *-Significant at $p \leq 0.05$; **-Significant at $p \leq 0.01$: Mean (±SE) bearing different superscripts within a column are significantly different at $p \leq 0.01$.

Table 4: Various mast cell tumors showing c-KIT under and overexpression

C-KIT overexpressed (≥ 28.01)				c-KIT under expressed (≤ 28.01)			
SL. No.	**Type of tumor**	**N**	**Mean± SE**	**SL. No.**	**Type of tumor**	**N**	**Mean± SE**
Patnaik grading system							
1	Grade I	0		1	Grade I	9	14.98±1.67
2	Grade II	7	47.55±4.65	2	Grade II	6	20.90±2.14
3	Grade III	8	79.22±3.98	3	Grade III	0	
Total		15		Total		15	
Kiupel grading system							
1	High	14	67.01±4.61	1	High	0	
2	Low	1	28.44	2	Low	15	17.35±1.49
	Total	15			Total		15

Table 5: Details of c-KIT expression in relation to postsurgical outcome in mast cell tumors

C-KIT	Number	Postsurgical outcome			
		Alive	**Recurrence**	**Dead**	**Total**
Overexpression	15	4	6	5	15
Under expression	15	13	1	1	15
Total	30	17	7	6	30

Table 5a: Kruskal Walli's test for comparison of mean (±SE) c-KIT values between various postsurgical outcome groups

Follow up data	Total number of cases	Mean (±SE) of c-KIT expression RT PCR (fold expression)
Alive	17	27.55±5.46[a]
Recurrence	7	59.29±8.47[b]
Dead	6	61.01±9.47[c]
Significance		*

Note: Kruskal-Walli's test: *-Significant at $p \leq 0.05$: Mean (±SE) bearing different superscripts within a column are significantly different at $p \leq 0.05$.

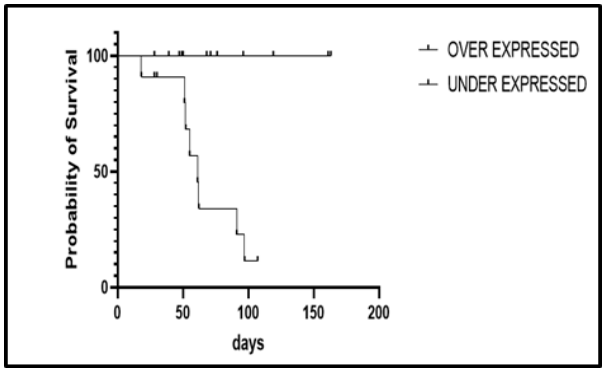

Fig 1: Kaplan-Meier survival curves for dogs with mast cell tumors grouped into overexpressed and underexpressed c-KIT profile ($p \leq 0.01$).

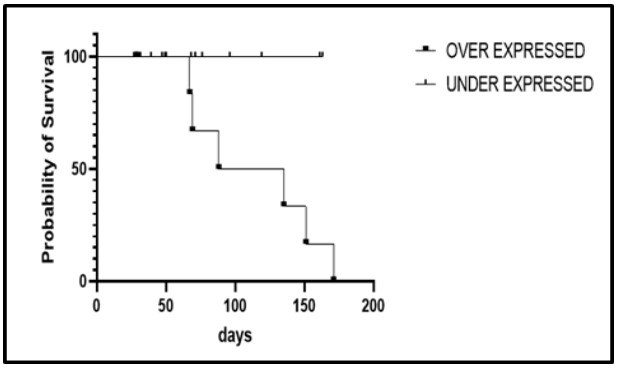

Fig 2: Kaplan-Meier curves for recurrence in dogs with mast cell tumors grouped into overexpressed and underexpressed c-KIT profile ($p \leq 0.05$).

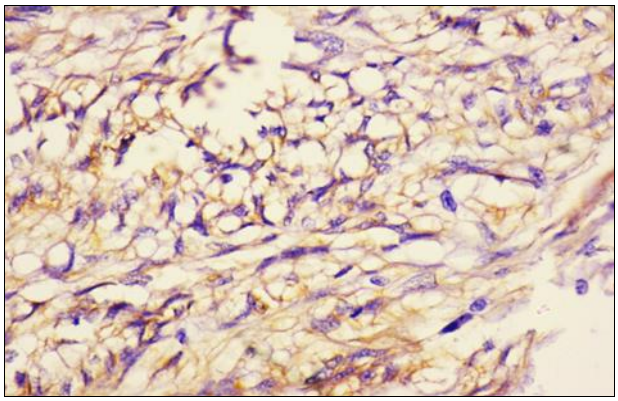

Fig 3: Histological section showing positive control, canine gastrointestinal stromal tumors showing c-KIT immunoreactivity: brown to dark brown cytoplasmic and membranous immunostaining (IHC X1000)

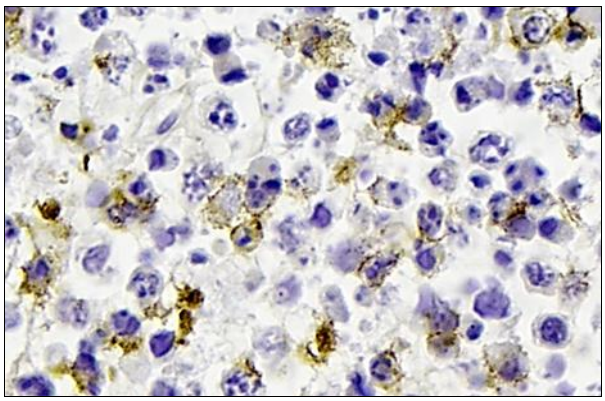

Fig 4: Histological section showing MCT c-KIT immunoreactivity classified as Pattern I characterized by membranous immunostaining (IHC X1000)

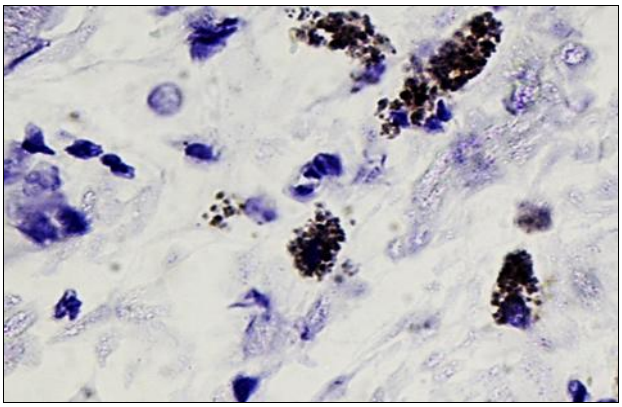

Fig 5: Histological section showing MCT c-KIT immunoreactivity classified as Pattern II characterized by focal/stippled cytoplasmic immunostaining (IHC X1000)

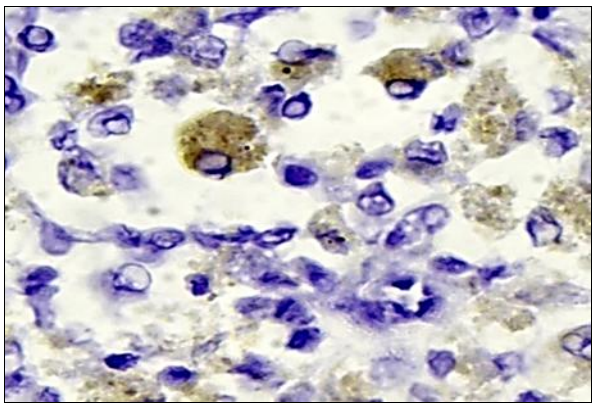

Fig 6: Histological section showing MCT c-KIT immunoreactivity classified as pattern III characterized by diffuse cytoplasmic immunostaining (IHC X1000)

Discussion

In the present study, immunohistochemical analysis of c-KIT protein in canine mast cell tumors (MCTs) revealed three distinct staining patterns, consistent with the classification by Kiupel *et al.* (2004) [8]. Pattern I, characterized by membranous localization; Pattern II, showing focal or stippled cytoplasmic staining with reduced membranous expression; and Pattern III, defined by diffuse cytoplasmic localization. Among the 30 tumors examined, 10 exhibited Pattern I, 16 Pattern II, and 4 Pattern III. Higher-grade tumors predominantly displayed aberrant cytoplasmic c-KIT expression (Patterns II and III), whereas membranous localization (Pattern I) was more common in low-grade tumors. This shift from membranous to cytoplasmic expression has been widely associated with increased cellular proliferation, higher recurrence rates, shorter survival, and poor prognosis (Kiupel *et al.*, 2004; Webster *et al.*, 2006; Gil da Costa *et al.*, 2007; Welle *et al.*, 2008; Thompson *et al.*, 2016; de Nardi *et al.*, 2022 and Darshan, 2025) [8, 17, 6, 4, 3]. These findings support the utility of c-KIT immunostaining as a prognostic marker in canine MCTs, providing valuable insights into tumor aggressiveness, recurrence risk, and informing clinical management strategies.

In the present study, real-time PCR analysis of 30 canine mast cell tumors revealed a wide range of c-KIT mRNA expression (7.49 to 92.73 folds) with a mean (± SE) of 40.90 ± 5.10. Expression levels correlated positively with tumor grade, with Patnaik Grade III tumors showing the highest overexpression,

followed by Grades II and I. Similarly, in the Kiupel grading system, high-grade tumors demonstrated significantly elevated c-KIT expression compared to low-grade tumors. Statistical analyses confirmed these differences as significant (Kruskal-Walli's test, $p \leq 0.05$; Mann-Whitney U test, $p \leq 0.01$), consistent with previous reports linking higher c-KIT expression to aggressive tumor behavior (Giantin *et al.*, 2012 and Darshan, 2025) [5, 3].

The prognostic relevance of c-KIT expression was assessed by correlating gene expression with post-surgical outcomes over a seven-month follow-up. Tumors were stratified using a median fold-change value (28.01) into overexpression and underexpression groups. Among the overexpression group, most cases were associated with recurrence or death, and Kaplan-Meier analysis showed significantly shorter survival (median 51.5 days) compared to the underexpression group, which largely remained disease-free. Similarly, higher c-KIT expression correlated with increased recurrence rates (median 110.1 days), indicating that overexpression is a predictor of poor postsurgical outcomes. Statistical evaluation using Kruskal-Wallis and log-rank tests confirmed the association between elevated c-KIT expression and adverse prognosis ($p \leq 0.05$-0.01), in agreement with previous studies (Zemke *et al.*, 2002; Webster *et al.*, 2006; Giantin *et al.*, 2012 and Darshan, 2025) [20, 17, 5, 3].

Although a few overexpressing tumors remained disease-free during the short follow-up, this may reflect the early stage of tumor progression or advanced presentation at diagnosis. These findings underscore c-KIT expression as a valuable prognostic marker in canine MCTs and suggest its potential role in selecting candidates for tyrosine kinase inhibitor therapy. However, further long-term studies are needed to validate c-KIT as an independent prognostic indicator and to clarify its mechanistic contribution to mast cell tumor progression.

Conclusion

This study demonstrates that c-KIT expression, assessed by both immunohistochemistry and real-time PCR, correlates strongly with tumor grade, post-surgical outcomes, and overall prognosis in canine mast cell tumors. Aberrant cytoplasmic c-KIT localization and mRNA overexpression were predominantly observed in high-grade tumors and were associated with increased recurrence, reduced survival, and poor prognosis, whereas lower expression correlated with disease-free outcomes. RT-PCR analysis confirmed upregulation of c-KIT mRNA in high-grade tumors, supporting its role in tumor aggressiveness. Elevated c-KIT expression correlated with recurrence and decreased survival, highlighting its prognostic utility. Statistical analyses, including Kruskal-Wallis tests and Kaplan-Meier survival curves, confirmed these associations, supporting the utility of c-KIT as a prognostic marker and a potential guide for tyrosine kinase inhibitor therapy. These findings underscore the value of integrating molecular and immunohistochemical c-KIT evaluation for comprehensive prognostic assessment in canine MCTs, with long-term studies needed to validate its role as an independent prognostic biomarker.

Conflict of Interest

Not available

Financial Support

Not available

Reference

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