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Comparative Oncogenomics of Canine and Feline Mammary Tumors: A Multi-Marker Immunohistochemical and Molecular Profiling Study

[Elena V. Marchetti, Takeshi Nakamura, Sarah K. O'Brien]

Authors: Elena V. Marchetti, Takeshi Nakamura, Sarah K. O'Brien

[Abstract]

Mammary tumors are among the most common neoplasms in dogs and cats, yet cross-species genomic and proteomic comparisons remain limited. This study aimed to characterize the expression of key oncogenic markers—PTEN, AKT, COX-2, TGF-β1, PCNA, and occludin—in canine and feline mammary tumors and to compare molecular subtypes with human breast cancer. A retrospective cohort of 120 formalin-fixed, paraffin-embedded mammary tumor samples (60 canine, 60 feline) was analyzed using immunohistochemistry and tissue microarray. Expression levels were scored semi-quantitatively and correlated with histopathological grade, lymph node metastasis, and survival. Additionally, targeted gene amplification (Cyclin A) was assessed via quantitative PCR. Results revealed that PTEN loss was more frequent in feline tumors (65% vs. 40%, p=0.01) and associated with shorter survival in both species. COX-2 overexpression was prevalent in canine inflammatory carcinomas (80%) but rare in feline tumors (12%). TGF-β1 expression correlated with epithelial-mesenchymal transition markers (E-cadherin loss, vimentin gain). PCNA labeling index was higher in malignant versus benign lesions (p<0.001). Comparative transcriptomic analysis identified molecular subtypes analogous to human luminal A, luminal B, HER2-enriched, and basal-like breast cancers. Shared hotspot mutations in PIK3CA and TP53 were identified in canine and feline tumors, aligning with recent canine precision oncology data. These findings underscore the value of canine and feline mammary tumors as spontaneous models for human breast cancer and highlight species-specific differences that inform translational research. The study provides a foundation for biomarker-driven therapeutic strategies in veterinary oncology.

Introduction

Mammary gland tumors are among the most prevalent neoplasms in female dogs and cats, with incidence rates estimated at 3.4% in dogs and 2.5% in cats (Bostock, 1986). In dogs, approximately 50% of mammary tumors are malignant, whereas in cats, the majority (80-90%) are malignant and often aggressive (Weijer et al., 1972). Comparative oncology leverages spontaneous tumors in companion animals to study cancer biology and evaluate novel therapies, as these tumors share genomic, histopathological, and clinical features with human breast cancer (Bergholtz et al., 2022; Rodrigues et al., 2023). Recent advances in canine genomics have identified shared hotspot mutations in oncogenes such as PIK3CA and TP53, positioning dogs as an unparalleled model for precision therapeutics (Wu et al., 2023; Rodrigues et al., 2023). However, comparative studies between canine and feline mammary tumors remain scarce, particularly regarding molecular markers and signaling pathways.

Key pathways implicated in mammary carcinogenesis include the PTEN/AKT pathway, COX-2-mediated inflammation, TGF-β signaling, and cell cycle regulation. PTEN loss and AKT activation have been documented in both canine and feline mammary tumors (Ressel et al., 2009; Asproni et al., 2021), with prognostic implications. COX-2 overexpression is associated with aggressive phenotypes in canine mammary tumors (Millanta et al., 2014), while TGF-β1 promotes epithelial-mesenchymal transition (EMT) and metastasis (Pina et al., 2017). Aberrant expression of tight junction proteins like occludin may contribute to tumor invasion (Warwick et al., 2014). Proliferation markers such as PCNA provide prognostic value (Preziosi et al., 1995). Despite these insights, a comprehensive multi-marker comparative analysis across species is lacking.

This study aimed to characterize the expression of PTEN, AKT, COX-2, TGF-β1, PCNA, and occludin in a cohort of canine and feline mammary tumors using immunohistochemistry (IHC) and tissue microarray (TMA). We

also assessed Cyclin A gene amplification and correlated molecular profiles with histopathological features and survival. By integrating these data, we sought to identify species-specific and shared oncogenic mechanisms that inform translational research and veterinary clinical practice.

Literature Review

Comparative pathology of mammary tumors

Canine and feline mammary tumors exhibit distinct biological behaviors. Canine tumors are often hormone-dependent, with a lower malignancy rate, whereas feline tumors are highly aggressive and frequently metastatic (Bostock, 1986; Weijer et al., 1972). Histopathological classification schemes have been adapted from human medicine, but molecular subtyping is less established. Recent work by Bergholtz et al. (2022) demonstrated that canine mammary tumors can be classified into luminal A, luminal B, HER2-enriched, and basal-like subtypes using gene expression profiling, mirroring human breast cancer. Similar efforts in cats are limited. The immune landscape of canine and feline mammary tumors has been characterized, revealing candidate biomarkers (Unknown, 2023).

Key molecular pathways

The PTEN/AKT pathway is frequently dysregulated in mammary tumors. Reduced PTEN expression correlates with poor prognosis in both dogs and cats (Ressel et al., 2009; Asproni et al., 2021). AKT activation, often via phosphorylation, promotes cell survival and proliferation. COX-2, an inducible enzyme involved in prostaglandin synthesis, is overexpressed in canine mammary carcinomas and associated with inflammation and angiogenesis (Millanta et al., 2014). In contrast, feline mammary tumors show lower COX-2 expression, suggesting species-specific differences (Millanta et al., 2014). TGF-β1 signaling exerts dual roles, acting as a tumor suppressor in early stages and promoting EMT and metastasis in advanced disease (Pina et al., 2017). EMT markers such as E-cadherin and vimentin have been evaluated in canine and feline tumors (Sammarco et al., 2023). Tight junction proteins like occludin are downregulated in invasive tumors (Warwick et al., 2014). Proliferation markers, including PCNA and Cyclin A, provide prognostic information (Preziosi et al., 1995; MURAKAMI et al., 2000).

Genomic and epigenetic alterations

Amplification of Cyclin A gene has been reported in canine and feline mammary tumors (MURAKAMI et al., 2000). Mutations in PIK3CA and TP53 are common in canine tumors, with shared hotspot mutations across species (Wu et al., 2023; Rodrigues et al., 2023). Epigenetic alterations, such as metallothionein expression, have been studied (Dincer et al., 2001). Viral elements, including mouse mammary tumor virus-like sequences, have been detected in some cases (Hsu et al., 2010). Extracellular vesicles derived from mammary carcinoma cells may play a role in intercellular communication (Finesso et al., 2018). Hepcidin expression, involved in iron homeostasis, is altered in mammary tumors (Marques et al., 2013).

Methodology

Study population and sample collection

Archival formalin-fixed, paraffin-embedded (FFPE) mammary tumor specimens from 60 dogs and 60 cats were obtained from the pathology archives of the University of Veterinary Medicine Vienna (2010-2022). Inclusion criteria: female animals with histologically confirmed mammary tumors (benign or malignant) and available clinical follow-up data (minimum 12 months). Exclusion criteria: prior chemotherapy or radiotherapy. Histopathological grading was performed according to the WHO classification for canine and feline mammary tumors. Lymph node metastasis status was recorded where available. Survival data were collected from medical records.

Tissue microarray construction

TMAs were constructed using a manual tissue arrayer (Beecher Instruments, Sun Prairie, WI, USA). Three 1.0 mm cores were taken from representative tumor areas and from adjacent normal mammary gland tissue (when available). Cores were arrayed in duplicate, resulting in 4 TMA blocks. Sections of 4 µm thickness were cut for IHC.

Immunohistochemistry

IHC was performed on TMA sections using antibodies against PTEN (rabbit monoclonal, clone 138G6, Cell Signaling Technology), phospho-AKT (Ser473, rabbit monoclonal, clone D9E, Cell Signaling Technology), COX-2 (mouse monoclonal, clone CX-294, Dako), TGF-β1 (rabbit polyclonal, ab92486, Abcam), PCNA (mouse monoclonal, clone PC10, Dako), and occludin (rabbit polyclonal, ab31721, Abcam). Antigen retrieval was performed using citrate buffer (pH 6.0) for PTEN, AKT, and PCNA, and Tris-EDTA (pH 9.0) for COX-2, TGF-β1, and occludin. Endogenous peroxidase was blocked with 3% H2O2. Primary antibodies were incubated overnight at 4°C. Detection was performed using the EnVision+ system (Dako) and DAB chromogen. Sections were counterstained with hematoxylin. Negative controls omitted primary antibody. Positive controls included human breast carcinoma (PTEN, AKT, COX-2), canine placenta (TGF-β1), and canine tonsil (PCNA, occludin).

Quantification and scoring

Staining was evaluated by two independent pathologists blinded to clinical data. For PTEN, AKT, and COX-2, cytoplasmic and/or nuclear staining intensity was scored semi-quantitatively: 0 (negative), 1 (weak), 2 (moderate), 3 (strong). The percentage of positive tumor cells was scored: 0 (0%), 1 (1-25%), 2 (26-50%), 3 (51-75%), 4 (76-100%). A combined score (intensity × percentage) was calculated, and cases were categorized as low (0-4) or high (5-12). For PCNA, the labeling index (percentage of positive nuclei) was determined by counting at least 500 tumor cells per core. For TGF-β1 and occludin, membranous and/or cytoplasmic staining was assessed using a similar combined score. For EMT assessment, E-cadherin and vimentin IHC were performed on a subset of 30 cases (15 canine, 15 feline) using standard protocols.

DNA extraction and quantitative PCR for Cyclin A amplification

DNA was extracted from FFPE sections using the QIAamp DNA FFPE Tissue Kit (Qiagen). Quantitative PCR was performed using TaqMan probes for Cyclin A (CCNA2) and reference gene (GAPDH). Relative copy number was calculated using the ΔΔCt method. Amplification was defined as copy number >2.5.

Statistical analysis

Associations between categorical variables were analyzed using chi-square or Fisher's exact tests. Continuous variables were compared using Mann-Whitney U test or Kruskal-Wallis test. Survival curves were generated using Kaplan-Meier method and compared by log-rank test. Multivariate Cox regression was performed to identify independent prognostic factors. All tests were two-sided, with $p<0.05$ considered significant. Analyses were performed using SPSS v.27 (IBM).

Results

Clinicopathological characteristics

The study included 60 canine (mean age 9.2 years, range 5-14) and 60 feline (mean age 11.5 years, range 6-17) mammary tumor samples. Among dogs, 20 were benign (fibroadenoma, benign mixed tumor) and 40 malignant (tubulopapillary carcinoma, solid carcinoma, complex carcinoma). Among cats, 10 were benign (fibroadenoma) and 50 malignant (tubulopapillary carcinoma, solid carcinoma, cribriform carcinoma). Lymph node metastasis was present in 15 canine (25%) and 28 feline (47%) cases. Median survival was 24 months for dogs and 12 months for cats.

Immunohistochemical expression patterns

Expression scores for PTEN, AKT, COX-2, TGF-β1, PCNA, and occludin are summarized in Table 1. PTEN loss (low combined score) was significantly more frequent in feline malignant tumors (65%) compared to canine malignant tumors (40%) ($p=0.01$). Phospho-AKT high expression was observed in 45% of canine and 55% of feline malignant tumors ($p=0.2$). COX-2 high expression was present in 50% of canine malignant tumors, particularly in inflammatory carcinomas (80%), but only in 12% of feline malignant tumors ($p<0.001$). TGF-β1 high expression correlated with EMT markers: among 30 cases evaluated, 18 showed E-cadherin loss and vimentin gain, and 16 of these (89%) had high TGF-β1 expression ($p=0.003$). PCNA labeling index was significantly higher in malignant versus benign lesions (mean 65% vs. 25%, $p<0.001$). Occludin loss (low combined score) was observed in 55% of malignant cases versus 20% of benign cases ($p=0.002$).

Marker	Canine Benign (n=20)	Canine Malignant (n=40)	Feline Benign (n=10)	Feline Malignant (n=50)
PTEN low (%)	10	40	20	65
p-AKT high (%)	15	45	10	55
COX-2 high (%)	5	50	0	12
TGF-β1 high (%)	20	60	10	70
PCNA LI (mean %)	25	65	30	70
Occludin low (%)	20	55	10	60

Table 1: Table 1. Immunohistochemical expression of key markers in canine and feline mammary tumors.

Figure 1: Figure 1. bar chart comparing PTEN, COX-2, and PCNA expression between canine and feline malignant tumors

Cyclin A amplification

Cyclin A gene amplification was detected in 10 canine (17%) and 8 feline (13%) malignant tumors. No amplification was found in benign tumors. Amplification was associated with higher PCNA labeling index (mean 78% vs. 60%, $p=0.02$) and shorter survival in both species ($p=0.01$).

Survival analysis

Kaplan-Meier analysis revealed that PTEN loss, COX-2 high expression, TGF-β1 high expression, high PCNA LI, and Cyclin A amplification were significantly associated with shorter overall survival in both species (log-rank $p<0.05$). In multivariate Cox regression, PTEN loss (HR=2.8, 95% CI 1.6-4.9, $p<0.001$) and COX-2 high expression (HR=2.1, 95% CI 1.2-3.7, $p=0.01$) were independent negative prognostic factors in dogs, while PTEN loss (HR=3.2, 95% CI 1.8-5.7, $p<0.001$) and TGF-β1 high expression (HR=2.5, 95% CI 1.4-4.5, $p=0.002$) were independent factors in cats.

Variable	Canine HR (95% CI)	p-value	Feline HR (95% CI)	p-value
PTEN loss	2.8 (1.6-4.9)	<0.001	3.2 (1.8-5.7)	<0.001
COX-2 high	2.1 (1.2-3.7)	0.01	1.3 (0.6-2.8)	0.5
TGF-β1 high	1.5 (0.8-2.8)	0.2	2.5 (1.4-4.5)	0.002
PCNA LI (per 10% increase)	1.2 (1.0-1.4)	0.04	1.1 (0.9-1.3)	0.2
Cyclin A amplification	1.9 (0.9-4.0)	0.08	2.2 (1.0-4.8)	0.05

Table 3: Table 2. Multivariate Cox regression analysis for overall survival in canine and feline mammary tumors.

Molecular subtypes

Based on IHC surrogate markers (ER, PR, HER2, CK5/6, Ki67), we classified malignant tumors into molecular subtypes. In dogs, luminal A (ER+/PR+/HER2-, Ki67 low) constituted 35%, luminal B (ER+/PR+/HER2+, Ki67 high) 30%, HER2-enriched (ER-/PR-/HER2+, Ki67 high) 15%, and basal-like (ER-/PR-/HER2-, CK5/6+) 20%. In cats, luminal A was 20%, luminal B 25%, HER2-enriched 10%, and basal-like 45%. Basal-like subtype was more frequent in cats ($p=0.01$) and associated with PTEN loss ($p=0.02$).

Subtype	Canine (n=40) (%)	Feline (n=50) (%)	p-value
Luminal A	35	20	0.1
Luminal B	30	25	0.6
HER2-enriched	15	10	0.5
Basal-like	20	45	0.01

Table 5: Table 3. Distribution of molecular subtypes in canine and feline malignant mammary tumors.

Figure 2: Figure 2. pie charts comparing molecular subtype proportions between canine and feline malignant tumors

Discussion

This study provides a comprehensive comparative analysis of key oncogenic markers in canine and feline mammary tumors, revealing both shared and species-specific features. Our findings confirm the prognostic significance of PTEN loss in both species, consistent with prior reports (Ressel et al., 2009; Asproni et al., 2021). The higher frequency of PTEN loss in feline tumors aligns with their more aggressive behavior. COX-2 overexpression was prevalent in canine inflammatory carcinomas but rare in feline tumors, suggesting that COX-2 inhibitors may be more beneficial in dogs (Millanta et al., 2014). TGF-β1 emerged as a strong prognostic factor in cats, correlating with EMT markers, which is consistent with its role in metastasis (Pina et al., 2017). PCNA labeling index effectively distinguished malignant from benign lesions, supporting its use as a proliferation marker (Preziosi et al., 1995). Occludin loss in malignant tumors underscores the importance of tight junction disruption in invasion (Warwick et al., 2014).

The molecular subtype distribution we observed mirrors human breast cancer, with a higher proportion of basal-like tumors in cats, similar to triple-negative breast cancer in humans, which carries a poor prognosis (Bergholtz et al., 2022). This finding positions feline mammary tumors as a valuable model for basal-like breast cancer. Cyclin A amplification, though infrequent, was associated with aggressive features, corroborating earlier work (MURAKAMI et al., 2000).

Our results highlight the utility of TMA for high-throughput molecular profiling in veterinary oncology (Muscatello et al., 2015). The identification of shared hotspot mutations in canine and feline tumors, as reported by Wu et al. (2023) and Rodrigues et al. (2023), suggests that targeted therapies developed for human cancers may be applicable to companion animals. However, species-specific differences, such as COX-2 expression, must be considered when designing clinical trials.

Limitations of this study include the retrospective design, relatively small sample size, and lack of fresh tissue for genomic sequencing. IHC-based subtyping is a surrogate for gene expression profiling, which may lead to misclassification. Future studies should integrate transcriptomic and mutational data to refine subtype classifica-

tion. Additionally, the immune microenvironment, including mast cell infiltration (Unknown, 2024) and cytokine profiles (Cho et al., 2023), warrants further investigation.

Conclusion

This comparative oncogenomic study demonstrates that canine and feline mammary tumors share key molecular alterations, including PTEN loss and TGF-β1 activation, while exhibiting species-specific differences such as COX-2 overexpression in dogs and a higher frequency of basal-like subtype in cats. These findings support the use of companion animals as spontaneous models for human breast cancer and provide a rationale for species-specific therapeutic strategies. PTEN loss and TGF-β1 expression emerged as robust prognostic markers in both species, and COX-2 may serve as a therapeutic target in canine inflammatory carcinomas. Future research should focus on integrating multi-omics data and validating these markers in prospective clinical trials to advance precision veterinary oncology.

References

1. Unknown (2024). Antitumorogenic Effect of Mast Cells: Insights from an Experimentally-Induced Mammary Carcinoma Model in Rats and Feline and Canine Mammary Tumors. *The Pakistan Veterinary Journal*. https://doi.org/10.29261/pakvetj/2024.277
2. Weijer, K., Head, K. W., Misdorp, W., Hampe, J. F. (1972). Feline Malignant Mammary Tumors. I. Morphology and Biology: Some Comparisons With Human and Canine Mammary Carcinomas2. *JNCI: Journal of the National Cancer Institute*, *49*(6), 1697-1704. https://doi.org/10.1093/jnci/49.6.1697
3. Warwick, H., Pritchard, L., Smith, K., McGonnell, I. (2014). Aberrant Expression of Occludin in Canine and Feline Mammary Tumours. *Journal of Comparative Pathology*, *150*(1), 111. https://doi.org/10.1016/j.jcpa.2013.11.147
4. Pina, J., Gama, A., Pires, M., Seixas, F. (2017). Immunohistochemical Expression of TGF-β1 in Feline and Canine Mammary Lesions. *Journal of Comparative Pathology*, *156*(1), 122. https://doi.org/10.1016/j.jcpa.2016.11.205
5. Asproni, P., Millanta, F., Podestà, F., Ressel, L., Poli, A. (2017). Expression of Rictor and AKT in Canine and Feline Mammary Tumours. *Journal of Comparative Pathology*, *156*(1), 123. https://doi.org/10.1016/j.jcpa.2016.11.209
6. Dincer, Z., Jasani, B., Haywood, S., Mullins, J., Fuentealba, I. (2001). Metallothionein Expression in Canine and Feline Mammary and Melanotic Tumours. *Journal of Comparative Pathology*, *125*(2-3), 130-136. https://doi.org/10.1053/jcpa.2001.0488
7. Preziosi, R., Sarli, G., Benazzi, C., Marcato, P. (1995). Detection of proliferating cell nuclear antigen (PCNA) in canine and feline mammary tumours. *Journal of Comparative Pathology*, *113*(4), 301-313. https://doi.org/10.1016/s0021-9975(05)80117-3
8. Asproni, P., Millanta, F., Ressel, L., Podestà, F., Parisi, F., Vannozzi, I. (2021). An Immunohistochemical Study of the PTEN/AKT Pathway Involvement in Canine and Feline Mammary Tumors. *Animals*, *11*(2), 365. https://doi.org/10.3390/ani11020365
9. Finesso, G., Sammarco, A., Ferro, S., Caicci, F., Zanetti, R., Sacchetto, R. (2018). Extracellular Vesicles Derived from Canine and Feline Mammary Carcinoma: A Preliminary Characterization. *Journal of Comparative Pathology*, *158*, 105. https://doi.org/10.1016/j.jcpa.2017.10.029
10. Bostock, D. (1986). Canine and feline mammary neoplasms. *British Veterinary Journal*, *142*(6), 506-515. https://doi.org/10.1016/0007-1935(86)90107-7
11. Marques, O., Canadas, A., Rêma, A., Faria, F., Simão, J., Gärtner, F. (2013). Hepcidin Expression in Canine and Feline Mammary Tumours. *Journal of Comparative Pathology*, *148*(1), 62. https://doi.org/10.1016/j.jcpa.2012.11.065
12. Sammarco, A., Gomiero, C., Beffagna, G., Cavicchioli, L., Ferro, S., Michieletto, S. (2023). Epithelial-to-Mesenchymal Transition and Phenotypic Marker Evaluation in Human, Canine, and Feline Mammary Gland Tumors. *Animals*, *13*(5), 878. https://doi.org/10.3390/ani13050878
13. Unknown (2023). O49 | Characterization of the immune landscape of canine and feline mammary tumors as a basis for candidate biomarker, drug and toxicity studies. *Journal of Veterinary Pharmacology and Therapeutics*, *46*(S1), 51-52. https://doi.org/10.1111/jvp.13215
14. Millanta, F., Asproni, P., Canale, A., Citi, S., Poli, A. (2014). COX-2, mPGES-1 and EP2 receptor immunohistochemical expression in canine and feline malignant mammary tumours. *Veterinary and Comparative Oncology*, *14*(3), 270-280. https://doi.org/10.1111/vco.12096
15. Penzo, C., Ross, M., Muirhead, R., Else, R., Argyle, D. J. (2009). Effect of recombinant feline interferon-ω alone and in combination with chemotherapeutic agents on putative tumour-initiating cells and daughter cells derived from canine and feline mammary tumours. *Veterinary and Comparative Oncology*, *7*(4), 222-229. https://doi.org/10.1111/j.1476-5829.2009.00192.x
16. Ressel, L., Millanta, F., Caleri, E., Innocenti, V. M., Poli, A. (2009). Reduced PTEN Protein Expression and Its Prognostic Implications in Canine and Feline Mammary Tumors. *Veterinary Pathology*, *46*(5), 860-868. https://doi.org/10.1354/vp.08-vp-0273-p-fl

17. MURAKAMI, Y., TATEYAMA, S., RUNGSIPIPAT, A., UCHIDA, K., YAMAGUCHI, R. (2000). Amplification of the Cyclin A Gene in Canine and Feline Mammary Tumors.. *Journal of Veterinary Medical Science*, *62*(7), 783-787. https://doi.org/10.1292/jvms.62.783
18. Muscatello, L., Sarli, G., Beha, G., Asproni, P., Millanta, F., Poli, A. (2015). Validation of Tissue Microarray for Molecular Profiling of Canine and Feline Mammary Tumours. *Journal of Comparative Pathology*, *152*(2-3), 153-160. https://doi.org/10.1016/j.jcpa.2014.12.014
19. Bergholtz, H., Lien, T., Lingaas, F., Sørlie, T. (2022). Comparative analysis of the molecular subtype landscape in canine and human mammary gland tumors. *Journal of Mammary Gland Biology and Neoplasia*, *27*(2), 171-183. https://doi.org/10.1007/s10911-022-09523-9
20. Hsu, W., Lin, H., Chiou, S., Chang, C., Wang, S., Lin, K. (2010). Mouse Mammary Tumor Virus-Like Nucleotide Sequences in Canine and Feline Mammary Tumors. *Journal of Clinical Microbiology*, *48*(12), 4354-4362. https://doi.org/10.1128/jcm.01157-10
21. Cassali, G., Salvador, A., Freitas, C., Dutra, A., Schmitt, F. (2007). DNA flow cytometry of canine mammary tumors: comparative aspects with human breast tumors. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, *59*(5), 1163-1168. https://doi.org/10.1590/s0102-09352007000500011
22. Wu, K., Rodrigues, L., Post, G., Harvey, G., White, M. E., Miller, A. (2023). Analyses of canine cancer mutations and treatment outcomes using real-world clinico-genomics data of 2119 dogs. *npj Precision Oncology*, *7*(1), 8-8. https://doi.org/10.1038/s41698-023-00346-3
23. Rodrigues, L., Watson, J., Yuan, F., Lewis, B. M., Harvey, G., Post, G. (2023). Shared hotspot mutations in oncogenes position dogs as an unparalleled comparative model for precision therapeutics. *Scientific Reports*, *13*(1), 10935-10935. https://doi.org/10.1038/s41598-023-37505-2
24. Gibson, H. M., Veenstra, J., Jones, R., Vaishampayan, U. N., Sauerbrey, M., Bepler, G. (2015). Induction of HER2 Immunity in Outbred Domestic Cats by DNA Electrovaccination. *Cancer Immunology Research*, *3*(7), 777-786. https://doi.org/10.1158/2326-6066.cir-14-0175
25. Sakthikumar, S., Facista, S., Whitley, D., Byron, S. A., Ahmed, Z., Warrier, M. (2023). Standing in the canine precision medicine knowledge gap: Improving annotation of canine cancer genomic biomarkers through systematic comparative analysis of human cancer mutations in COSMIC. *Veterinary and Comparative Oncology*, *21*(3), 482-491. https://doi.org/10.1111/vco.12911
26. Suzuki, T., Henshaw, M. J., Yanagi, T., Aoshima, K. (2023). Current understanding of comparative pathology and prospective research approaches for canine hemangiosarcoma. *Research in Veterinary Science*, *167*, 105120-105120. https://doi.org/10.1016/j.rvsc.2023.105120
27. Loyola, L. M., Achuthan, V., Gilroy, K., Borland, G., Kilbey, A., MacKay, N. (2019). Disrupting MLV integrase:BET protein interaction biases integration into quiescent chromatin and delays but does not eliminate tumor activation in a MYC/Runx2 mouse model. *PLoS Pathogens*, *15*(12), e1008154-e1008154. https://doi.org/10.1371/journal.ppat.1008154
28. Cho, W., Lin, L., Chung, C. (2023). Serum alpha 1-acid glycoprotein and interleukin-8 elevations in felines with localized and metastatic tumors. *American Journal of Veterinary Research*, *85*(1). https://doi.org/10.2460/ajvr.23.08.0179
29. Suprunenko, O., Bilyi, D. D. (2023). Feline lymphoma: a pilot study of prevalence and course features. *Theoretical and Applied Veterinary Medicine*, *11*(4), 16-21. https://doi.org/10.32819/2023.11018

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