

[Redacted]

Accession: [Redacted]
Received: [Redacted]
Reported: [Redacted]

Phone Number: [Redacted]
Account Number: [Redacted]



Pet: [Redacted] Owner: [Redacted]

Species: **Canine** Breed: [Redacted]

Sex: [Redacted] Age: [Redacted]

Site: **L mand. LN**

Diagnosis: **Lymphoma**

SearchLight DNA Overview

6 Biomarker(s) identified in the following genes:


KDR	KIT
MYC	PDGFRA
RB1	TRAF3


Sample QC Metrics


Specimen Type: FNACyto
Tumor Content (>20%): 95%
Mean Target Coverage (>200x): 439x

Number of Clinical Trials:

- **This Cancer Type:** 19
- **General Cancer:** 18

 6 Diagnostic Biomarkers

 3 Prognostic Biomarkers

 2 Matching Drugs: dasatinib, imatinib

SearchLight DNA Summary

An integrated review of the genomic data, as well as clinical history and pathology review, supports the diagnosis of lymphoma. Specifically, TRAF3 inactivating mutations (e.g. Val536fs), as well as copy number gains of KIT, KDR, PDGFRA and MYC, have been frequently found in canine and/or human lymphoma, especially B-cell lymphoma. While the genomic mutation profile is consistent with the diagnosis of B-cell lymphoma, further tests, such as IHC, flow, or PARR, could support additional phenotyping.

Notably, we identified mutations with therapeutic and prognostic associations based on FDA approval or well-powered studies in humans and/or dogs, as described on page 2. Dasatinib and imatinib are available through veterinary compounding pharmacies. Monographs describing published data on the use of these agents in dogs are available upon request, or you can find them on our website (<https://vidiumah.com/monographs/>).

This test evaluated 120 cancer genes in the submitted sample. The ABCB1-1delta (MDR1-1delta) mutation was not detected, indicating that the patient is unlikely to experience the ABCB1-1delta-related adverse effects of chemotherapy.



Therapeutic Biomarkers

Treatment Options Based on Mutations

Drug	Mutation	Available for dogs	Used in humans
dasatinib	PDGFRA Copy Number Gain	Yes	Yes ^d
imatinib	PDGFRA Copy Number Gain	Yes	Yes ^d

Drug Resistance-Associated Biomarkers

Drug	Mutation
-	-

Pharmacogenomic Biomarkers

Gene	Mutation
ABCB1	No ABCB1-1Δ Mutation



Diagnostic Biomarkers

Described in:

Gene	Mutation	Canine cancer	Human cancer
KDR	Copy Number Gain	Lymphoma ^b , Hemangiosarcoma ^b , Lung Carcinoma ^b , Mammary Cancer ^b	Yes ^b
KIT	Copy Number Gain	Lymphoma ^b , B-Cell Lymphoma ^b , Hemangiosarcoma ^b , Lung Carcinoma ^b , Mammary Cancer ^b , Mast Cell Tumor ^b , Melanoma ^b , T-Cell Lymphoma ^b	Yes ^b
MYC	Copy Number Gain	Lymphoma ^b , B-Cell Lymphoma ^b , Glioma ^b , Head and Neck Squamous Cell Carcinoma ^b , Hemangiosarcoma ^b , Mammary Cancer ^b , Melanoma ^b , Osteosarcoma ^b , T-Cell Lymphoma ^b	Yes ^b
PDGFRA	Copy Number Gain	Lymphoma ^b , Glioma ^b , Hemangiosarcoma ^b , Osteosarcoma ^b	Yes ^b
RB1	Copy Number Loss	Histiocytic Sarcoma ^b , Mammary Cancer ^b , Mast Cell Tumor ^b , Melanoma ^b , Osteosarcoma ^b	Yes ^b
TRAF3	p.Val536fs	Lymphoma ^b , B-Cell Lymphoma ^b	Yes ^b



Prognostic Biomarkers

Negative Prognostic Factor in:

Gene	Mutation	Canine cancer	Human cancer
MYC	Copy Number Gain	-	Yes ^b
PDGFRA	Copy Number Gain	-	Yes ^b
RB1	Copy Number Loss	-	Yes ^b

Evidence Level Key

- a** Validated biomarker - Proven biomarker with wide consensus and whose use is included in professional guidelines
- b** Clinical evidence - Biomarker with consensus from experts in the field with data obtained from large, well-powered studies
- c** Case studies - Biomarker suggested by data from one or more individual case reports from clinical journals
- d** Preclinical evidence - Biomarker suggested by data from *in vivo* or *in vitro* models

Mutation Summaries

Pet Name: XXXXXXXXXX

Gene	ABCB1	Mutation	No ABCB1-1Δ Mutation
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Variant Summary:

A polymorphism (referred to as ABCB1-1delta) occurs in a subset of dog breeds, including many herding breeds. The ABCB1-1delta polymorphism is a 4-base pair deletion that causes a shift in the reading frame that results in premature truncation of P-glycoprotein and loss of P-glycoprotein function. Dogs that are homozygous or heterozygous for this polymorphism can experience increased toxicity for chemotherapeutic agents that are substrates for ABCB1, such as doxorubicin, vincristine, and vinblastine. Dogs without this polymorphism (non-mutant) show standard susceptibility to chemotherapy-associated adverse effects, and a dosing adjustment based on ABCB1 status is not needed. (Mealey et al. J Vet Intern Med 2008; Mealey et al. Vet Clin North Am Small Anim Pract 2013)

Detailed Summary:

Please see [Link](#) for a detailed summary of this gene as well as information regarding this variant and its associated canine and human data.

Gene	KDR	Mutation	Copy Number Gain
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Roles in this case:  Diagnostic

Variant Summary:

The gene KDR encodes for the "Kinase Insert Domain Receptor" protein, a type III receptor tyrosine kinase and one of the two receptors of the VEGF, which is a major growth factor for endothelial cells. It functions as the main mediator of VEGF-induced endothelial proliferation, survival, migration, tubular morphogenesis and sprouting. It is an oncogene that is frequently activated via oncogenic mutations or copy number gains in cancer. KDR has been mutated in canine lymphoma (58%), hemangiosarcoma (21.7-28%), and lung carcinoma (20%). KDR copy number gains have been observed in canine B-cell lymphoma (58%), hemangiosarcoma (21.7-28%), lung carcinoma (20%). KDR copy number gains have been seen in 1.3% of human cancers, including glioblastoma, lung, and sarcoma cancers, among others. (COSMIC, TCGA Pan-Cancer Atlas accessed via cBioPortal).

Detailed Summary:

Please see [Link](#) for a detailed summary of this gene as well as information regarding this variant and its associated canine and human data.

Gene	KIT	Mutation	Copy Number Gain
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Roles in this case:  Diagnostic



Variant Summary:

The gene KIT encodes for the "KIT Proto-Oncogene, Receptor Tyrosine Kinase" protein, a transmembrane RTK. It is activated through dimerization and autophosphorylation upon binding by its ligands, which results in increased intracellular signaling through several pathways including PI3K, MAPK and STAT, ultimately leading to cell proliferation and survival. It is an oncogene that is frequently activated via oncogenic mutations or copy number gains in cancer. KIT has been mutated in canine melanoma (10-100%), mast cell tumor (MCT, 0.52-75%) , lymphoma (29.7-58%), gastrointestinal stromal tumor (2.17-41.3%), hemangiosarcoma (7.7-27%), lung carcinoma (20%), leukemia (9.8%), and melanoma (10%). KIT copy number gains have been observed in canine B-cell lymphoma (39.3-58%), melanoma (27-35%), T-cell lymphoma (29.7%), hemangiosarcoma (17.4-27%), lung carcinoma (20%). KIT copy number gains have been seen in 2% of human cancers, including glioblastoma, lung, and sarcoma cancers, among others. (COSMIC, TCGA Pan-Cancer Atlas accessed via cBioPortal).

Detailed Summary:

Please see [Link](#) for a detailed summary of this gene as well as information regarding this variant and its associated canine and human data.

Gene	MYC	Mutation	Copy Number Gain
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Roles in this case:  Diagnostic  Prognostic

Variant Summary:

The gene MYC encodes for the "MYC Proto-Oncogene, Bhlh Transcription Factor" protein, a nuclear phosphoprotein that plays a role in cell cycle progression, apoptosis and cellular transformation. The encoded protein forms a heterodimer with the related transcription factor MAX. This complex binds to the E box DNA consensus sequence and regulates the transcription of specific target genes. It is an oncogene that is frequently activated via oncogenic mutations or copy number gains in cancer. MYC has been mutated in canine osteosarcoma (33-85%), melanoma (80%), lymphoma (1-75%), squamous cell carcinoma (58%), mammary cancer (18.6-25%), glioma (14%), and hemangiosarcoma (6.7-8.7%). MYC copy number gains have been observed in canine osteosarcoma (33-85%), melanoma (80%), b-cell lymphoma (38.9-75%), squamous cell carcinoma (58%), t-cell lymphoma (29.7%), mammary cancer (18.6-25%), glioma (14%), hemangiosarcoma (8.7%). MYC copy number gains have been seen in 8.7% of human cancers, including ovarian, esophageal, and uterine cancers, among others. (COSMIC, TCGA Pan-Cancer Atlas accessed via cBioPortal). MYC copy number gains are a negative prognostic biomarker in human bladder, breast, colorectal, esophageal, gastric, hepatocellular carcinoma, lung, lymphoma, pancreatic, and prostate cancers.

Detailed Summary:

Please see [Link](#) for a detailed summary of this gene as well as information regarding this variant and its associated canine and human data.

Gene	PDGFRA	Mutation	Copy Number Gain
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Roles in this case:  Diagnostic  Prognostic  Therapeutic

Variant Summary:

The gene PDGFRA encodes for the "Platelet Derived Growth Factor Receptor Alpha" protein, a cell surface tyrosine kinase receptor for members of the platelet-derived growth factor family. Binding of ligand to the extracellular domain of PDGFRA causes dimerization followed by autophosphorylation of the receptor and activation of downstream pathways such as RAS-MAPK, PI3K and PLC- γ that are involved in organ development, wound healing, and tumor progression. It is an oncogene that is frequently activated via oncogenic mutations or copy number gains in cancer. PDGFRA has been mutated in canine lymphoma (58%), osteosarcoma (4-21%), hemangiosarcoma (7.7-20%), glioma (4%), and mammary cancer (0.5%). PDGFRA copy number gains have been observed in canine b-cell lymphoma (58%), osteosarcoma (21%), hemangiosarcoma (20%), glioma (4%). PDGFRA copy number gains have been seen in 1.7% of human cancers, including glioblastoma, lung, and sarcoma, among others. (COSMIC, TCGA Pan-Cancer Atlas accessed via cBioPortal). PDGFRA copy number gains are a negative prognostic biomarker in human glioma. PDGFRA copy number gains are associated with treatment sensitivity to dasatinib (a kinase inhibitor) in human melanoma.

Detailed Summary:

Please see [Link](#) for a detailed summary of this gene as well as information regarding this variant and its associated canine and human data.

Gene	RB1	Mutation	Copy Number Loss
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Roles in this case:  Diagnostic  Prognostic


Variant Summary:

The gene RB1 encodes for the "RB Transcriptional Corepressor 1" protein. The protein encoded by this gene is a negative regulator of the cell cycle and has roles in cell differentiation, survival, senescence, epigenetic regulation, and genome stability. It is a tumor suppressor gene (the first to be discovered), and is inactivated either via deletions (copy number loss) or loss-of-function mutations in cancer. RB1 has been mutated in canine histiocytic sarcoma (55.8%), mammary cancer (9.7-50%), osteosarcoma (2.7-50%), melanoma (3.08-35%), and mast cell tumor (18.2%). RB1 copy number losses have been observed in canine histiocytic sarcoma (55.8%), mammary cancer (9.7-50%), osteosarcoma (29-50%), melanoma (30.9-35%), mast cell tumor (18.2%). RB1 copy number losses have been seen in 5.1% of human cancers, including sarcoma, prostate, and uterine cancers, among others. (COSMIC, TCGA Pan-Cancer Atlas accessed via cBioPortal). RB1 copy number losses are a negative prognostic biomarker in human prostate cancer.

Detailed Summary:

Please see [Link](#) for a detailed summary of this gene as well as information regarding this variant and its associated canine and human data.

Gene	TRAF3	Mutation	p.Val536fs
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Roles in this case:  Diagnostic

Variant Summary:

The gene TRAF3 encodes for the "TNF Receptor Associated Factor 3" protein, a member of the TNF receptor associated factor (TRAF) protein family of proteins. The encoded protein is known to mediate signal transduction of CD40, a TNFR family member important for the activation of the immune response. It is a tumor suppressor gene and is inactivated either via deletions (copy number loss) or loss-of-function mutations in cancer. TRAF3 has been mutated in canine lymphoma (20-53.2%). TRAF3 loss-of-function mutations have been observed in canine lymphoma (20-46.7%). TRAF3 has been found to be mutated in 1.3% of human cancers, including uterine, melanoma, cervical, and other cancers. (COSMIC, TCGA Pan-Cancer Atlas accessed via cBioPortal).

Detailed Summary:

Please see [Link](#) for a detailed summary of this gene as well as information regarding this variant and its associated canine and human data.

Clinical Trials Summary

Pet Name: XXXXXXXXXX

Clinical Trial for this tumor type	Location	Website
AAHSD005097 - Efficacy of Rabafosadine, Vincristine, Cyclophosphamide, Doxorubicin and Prednisone (T-CHOP) in Dogs with Untreated Lymphoma.	Oregon State University Corvallis, OR	Link
AAHSD005199 - Study to Evaluate the Safety and Efficacy of Rabacfosadine, Vincristine, Cyclophosphamide, Doxorubicin and Prednisone in Dogs with Untreated Lymphoma	Colorado State University Fort Collins, CO	Link
AAHSD005330 - F18 FDG PET/CT and Cytology for Canine Lymphoma	Colorado State University Fort Collins, CO	Link
AAHSD005376 - AIM--Ablative Immune Modification with Nanopulse Stimulation Prior to Treatment with Doxorubicin in Dogs with Diffuse Large B-Cell Lymphoma.	Virginia-Maryland Regional College of Veterinary Medicine Blacksburg, VA	Link
AAHSD005421 - Glutathione-S-Transferase Polymorphisms in Dogs	University of Wisconsin Madison, WI	Link
ACI Lymphoma - A Multi-Center Pivotal Study to Determine the Effectiveness and Safety of Verdinexor for the Treatment of Lymphoma in Dogs	Animal Clinical Investigation, LLC Malvern, PA Denver, NC	Link
COSU-FACC Lymphoma 1 - Drug Repurposing to Aid Treatment of Canine Lymphoma	Cornell University - College of Veterinary Medicine Ithaca, NY	Link
COSU-FACC Lymphoma 2 - Evaluating a New Drug Combination for Dogs with Lymphoma	Cornell University - College of Veterinary Medicine Ithaca, NY	Link
HVS Lymphoma - Evaluating the Safety and Efficacy of TANOVEA?-CA1 When Combined with Vincristine, Cyclophosphamide, Doxorubicin and Prednisone (T-CHOP) in Dogs with Untreated Lymphoma	Hope Veterinary Specialists Malvern, PA	Link
OSU-CCVM Lymphoma - Canine Naive Multicentric Lymphoma Study	Oregon State University - Carlson College of Veterinary Medicine Corvallis, OR	Link
PU-CVM B-cell Lymphoma - Biodynamic Imaging (BDI) as a Promising Strategy for Personalized Therapy of Canine Diffuse Large B-cell Lymphoma	Purdue University - College of Veterinary Medicine West Lafayette, IN	Link
PU-CVM T-cell Lymphoma - Biodynamic Testing of Chemotherapy Sensitivity in Dogs Receiving Gemcitabine for Cutaneous T-cell Lymphoma	Purdue University - College of Veterinary Medicine West Lafayette, IN	Link
TAM-CVMBS Lymphoma - Use of a novel agent, RTI, to extend the disease free interval in dogs with lymphoma.	Texas A&M University - College of Veterinary Medicine & Biomedical Sciences College Station, TX	Link
UCD-SVM Lymphoma - Assessing Whether a Wearable Activity Monitor Can Improve Monitoring of Dogs with Lymphoma.	University of California-Davis - School of Veterinary Medicine Davis, CA	Link
UI-CVM Lymphoma - Assessing the Efficacy and Tolerability of Daunomustine in Dogs with Multicentric Lymphoma	University of Illinois - College of Veterinary Medicine Urbana, IL	Link
UPENN-SVM Lymphoma - Evaluation of Bendamustine as a Novel Chemotherapy Agent for Relapsed Canine Lymphoma	University of Pennsylvania - School of Veterinary Medicine Philadelphia, PA	Link

UWI-VC Lymphoma 1 - Effectiveness and safety of verdinexor for the treatment of Lymphoma in dogs	University of Wisconsin - Veterinary Care Madison, WI	Link
UWI-VC Lymphoma 2 - Evaluation of Laverdia (new drug) in combination with CCNU	University of Wisconsin - Veterinary Care Madison, WI	Link
VM-CVM Lymphoma - THOP chemotherapy protocol as front-line treatment of canine B-cell lymphoma	Virginia-Maryland - College of Veterinary Medicine Blacksburg, VA	Link

Other Clinical Trials That May Be Applicable

18 identified	See link for details
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Variants of Uncertain Significance

The following variants were detected in Kam Bell's tumor sample. These variants are considered variants of uncertain significance, meaning the functional impact of the alteration on gene function is unknown or the role of the mutation in tumor diagnosis, prognosis, or treatment is unknown. Future research may reveal a role for the mutations in cancer.

Variants of Uncertain Significance

References

1. Abida W et al. Genomic correlates of clinical outcome in advanced prostate cancer. *Proc Natl Acad Sci U S A* (2019). <https://pubmed.ncbi.nlm.nih.gov/31061129>
2. Alentorn A et al. Prevalence, clinico-pathological value, and co-occurrence of PDGFRA abnormalities in diffuse gliomas. *Neuro Oncol* (2012). <https://pubmed.ncbi.nlm.nih.gov/23074200>
3. Amin SB et al. Comparative Molecular Life History of Spontaneous Canine and Human Gliomas. *Cancer Cell* (2020). <https://pubmed.ncbi.nlm.nih.gov/32049048>
4. Angstadt AY et al. Characterization of canine osteosarcoma by array comparative genomic hybridization and RT-qPCR: signatures of genomic imbalance in canine osteosarcoma parallel the human counterpart. *Genes Chromosomes Cancer* (2011). <https://pubmed.ncbi.nlm.nih.gov/21837709>
5. Arico A et al. Array-based comparative genomic hybridization analysis reveals chromosomal copy number aberrations associated with clinical outcome in canine diffuse large B-cell lymphoma. *PLoS One* (2014). <https://pubmed.ncbi.nlm.nih.gov/25372838>
6. Beck J et al. Genome aberrations in canine mammary carcinomas and their detection in cell-free plasma DNA. *PLoS One* (2013). <https://pubmed.ncbi.nlm.nih.gov/24098698>
7. Borge KS et al. Canine Mammary Tumours Are Affected by Frequent Copy Number Aberrations, including Amplification of MYC and Loss of PTEN. *PLoS One* (2015). <https://pubmed.ncbi.nlm.nih.gov/25955013>
8. Brocca G et al. KIT Somatic Mutations and Immunohistochemical Expression in Canine Oral Melanoma. *Animals (Basel)* (2020). <https://pubmed.ncbi.nlm.nih.gov/33321993>
9. Bushell KR et al. Genetic inactivation of TRAF3 in canine and human B-cell lymphoma. *Blood* (2015). <https://pubmed.ncbi.nlm.nih.gov/25468570>
10. Chu S et al. Unraveling the chaotic genomic landscape of primary and metastatic canine appendicular osteosarcoma with current sequencing technologies and bioinformatic approaches. *PLoS One* (2021). <https://pubmed.ncbi.nlm.nih.gov/33556121>
11. Das S et al. Immune pathways and TP53 missense mutations are associated with longer survival in canine osteosarcoma. *Commun Biol* (2021). <https://pubmed.ncbi.nlm.nih.gov/34635775>
12. Eivers I et al. Exome sequencing of lymphomas from three dog breeds reveals somatic mutation patterns reflecting genetic background. *Genome Res* (2015). <https://pubmed.ncbi.nlm.nih.gov/26377837>
13. Gardner HL et al. Canine osteosarcoma genome sequencing identifies recurrent mutations in DMD and the histone methyltransferase gene SETD2. *Commun Biol* (2019). <https://pubmed.ncbi.nlm.nih.gov/31341965>
14. Giannuzzi D et al. The genomic landscape of canine diffuse large B-cell lymphoma identifies distinct subtypes with clinical and therapeutic implications. *Lab Anim* (2022). <https://pubmed.ncbi.nlm.nih.gov/35726023>
15. Hammerman PS et al. Mutations in the DDR2 kinase gene identify a novel therapeutic target in squamous cell lung cancer. *Cancer Discov* (2011). <https://pubmed.ncbi.nlm.nih.gov/22328973>
16. Hedan B et al. Molecular cytogenetic characterization of canine histiocytic sarcoma: A spontaneous model for human histiocytic cancer identifies deletion of tumor suppressor genes and highlights influence of genetic background on tumor behavior. *BMC Cancer* (2011). <https://pubmed.ncbi.nlm.nih.gov/21615919>
17. Hendricks WPD et al. Somatic inactivating PTPRJ mutations and dysregulated pathways identified in canine malignant melanoma by integrated comparative genomic analysis. *PLoS Genet* (2018). <https://pubmed.ncbi.nlm.nih.gov/30188888>
18. Holtkamp N et al. Mutation and expression of PDGFRA and KIT in malignant peripheral nerve sheath tumors, and its implications for imatinib sensitivity. *Carcinogenesis* (2006). <https://pubmed.ncbi.nlm.nih.gov/16357008>
19. Huang J et al. Prognostic Significance of c-MYC Amplification in Esophageal Squamous Cell Carcinoma. *Ann Thorac Surg* (2019). <https://pubmed.ncbi.nlm.nih.gov/30273571>
20. Lee KS et al. c-MYC Copy-Number Gain Is an Independent Prognostic Factor in Patients with Colorectal Cancer. *PLoS One* (2015). <https://pubmed.ncbi.nlm.nih.gov/26426996>
21. Liu D et al. Canine spontaneous head and neck squamous cell carcinomas represent their human counterparts at the molecular level. *PLoS Genet* (2015). <https://pubmed.ncbi.nlm.nih.gov/26030765>
22. Liu W et al. DNA alterations in the tumor genome and their associations with clinical outcome in prostate cancer. *Asian J Androl* (2016). <https://pubmed.ncbi.nlm.nih.gov/26975494>
23. Lorch G et al. Identification of Recurrent Activating HER2 Mutations in Primary Canine Pulmonary Adenocarcinoma. *Clin Cancer Res* (2019). <https://pubmed.ncbi.nlm.nih.gov/31431454>
24. Megquier K et al. Comparative Genomics Reveals Shared Mutational Landscape in Canine Hemangiosarcoma and Human Angiosarcoma. *Mol Cancer Res* (2019). <https://pubmed.ncbi.nlm.nih.gov/31570656>
25. Megquier K et al. The genomic landscape of canine osteosarcoma cell lines reveals conserved structural complexity and pathway alterations. *PLOS one* (2022). <https://pubmed.ncbi.nlm.nih.gov/36099278>
26. Mochizuki H et al. Genomic profiling of canine mast cell tumors identifies DNA copy number aberrations associated with KIT

- mutations and high histological grade. *Chromosome Res* (2017). <https://pubmed.ncbi.nlm.nih.gov/28058543>
27. Nedeljkovic M et al. Importance of Copy Number Alterations of FGFR1 and C-MYC Genes in Triple Negative Breast Cancer. *J Med Biochem* (2019). <https://pubmed.ncbi.nlm.nih.gov/30820185>
28. Poorman K et al. Comparative cytogenetic characterization of primary canine melanocytic lesions using array CGH and fluorescence in situ hybridization. *Chromosome Res* (2015). <https://pubmed.ncbi.nlm.nih.gov/25511566>
29. Schieppati F et al. An increase in MYC copy number has a progressive negative prognostic impact in patients with diffuse large B-cell and high-grade lymphoma, who may benefit from intensified treatment regimens. *Haematologica* (2020). <https://pubmed.ncbi.nlm.nih.gov/31399522>
30. Seo AN et al. Clinicopathologic and prognostic significance of c-MYC copy number gain in lung adenocarcinomas. *Br J Cancer* (2014). <https://pubmed.ncbi.nlm.nih.gov/24809777>
31. Smith PAD et al. The prevalence and characterisation of TRAF3 and POT1 mutations in canine B-cell lymphoma. *Vet J* (2020). <https://pubmed.ncbi.nlm.nih.gov/33323169>
32. Thomas R et al. Genomic profiling reveals extensive heterogeneity in somatic DNA copy number aberrations of canine hemangiosarcoma. *Chromosome Res* (2014). <https://pubmed.ncbi.nlm.nih.gov/24599718>
33. Thomas R et al. Refining tumor-associated aneuploidy through 'genomic recoding' of recurrent DNA copy number aberrations in 150 canine non-Hodgkin lymphomas. *Leuk Lymphoma* (2011). <https://pubmed.ncbi.nlm.nih.gov/21375435>
34. Wang X et al. Recurrent amplification of MYC and TNFRSF11B in 8q24 is associated with poor survival in patients with gastric cancer. *Gastric Cancer* (2016). <https://pubmed.ncbi.nlm.nih.gov/25618371>
35. Witkiewicz AK et al. Whole-exome sequencing of pancreatic cancer defines genetic diversity and therapeutic targets. *Nat Commun* (2015). <https://pubmed.ncbi.nlm.nih.gov/25855536>
36. Wong K et al. Cross-species genomic landscape comparison of human mucosal melanoma with canine oral and equine melanoma. *Nat Commun* (2019). <https://pubmed.ncbi.nlm.nih.gov/30664638>
37. Yu MC et al. Prediction of early-stage hepatocellular carcinoma using OncoScan chromosomal copy number aberration data. *World J Gastroenterol* (2017). <https://pubmed.ncbi.nlm.nih.gov/29209123>
38. Zaharieva B et al. High-throughput tissue microarray analysis of CMYC amplification in urinary bladder cancer. *Int J Cancer* (2005). <https://pubmed.ncbi.nlm.nih.gov/15986448>
39. Zhu S et al. Genetic alterations of TRAF proteins in human cancers. *Front Immunol* (2018). <https://pubmed.ncbi.nlm.nih.gov/30294322>

Additional Supporting Information

1. Alteration frequencies in human cancers are derived from COSMIC <https://cancer.sanger.ac.uk/> and the TCGA pan-cancer cohort, as accessed through cBioPortal <https://www.cbioportal.org/>
2. Gene summaries are based on gene descriptions provided by the National Library of Medicine and National Center for Biotechnology Information <https://www.ncbi.nlm.nih.gov/gene>
3. Mealey et al. ABCB1-1 Delta polymorphism can predict hematologic toxicity in dogs treated with vincristine. *J Vet Intern Med* (2008). <https://pubmed.ncbi.nlm.nih.gov>
4. Mealey et al. Adverse drug reactions in veterinary patients associated with drug transporters. *Vet Clin North Am Small Anim Pract* (2013). <https://pubmed.ncbi.nlm.nih.gov/23890239>

Genes Evaluated by SearchLight DNA

Pet Name: XXXXXXXXXX

SearchLight DNA detects multiple types of gene mutations:

- Single nucleotide variants, small nucleotide insertions and deletions (SNVs) occurring in selected commonly mutated regions in oncogenes ("Selected Exons") or in any coding region of a tumor suppressor gene ("All Coding Exons").
- Copy number variants (CNVs) including copy number gains encompassing oncogenes and copy number losses encompassing tumor suppressor genes.
- Internal tandem duplications (ITDs) occurring in oncogenes.
- Pharmacogenomic variants in genes that regulate drug processing.

	ARCB1	AKT1	AKT3	ALK	APC	ARAF	ARID1A	ASXL1	ATM	ATR	ATRX	BAP1	BRAF	BRCA1	BRCA2	BTX	CALR	CBL	CCND1	CCND2	CCND3	CCNE1	CDK12	CDK4	
SNV Selected Exons		•	•	•		•		•	•				•				•	•	•						
SNV All Coding Exons					•		•	•	•		•		•	•									•		
CNV		•			•		•	•	•	•	•	•	•	•					•	•	•	•	•	•	•
ITD																									
Pharmacogenomic	•																								

	CDK6	CDKN2A	CDKN2B	CHIE2	CRKL	CSF3R	CTNNB1	DDR2	DNMT3A	EGFR	ERBB2	ERBB3	ESR1	EZH2	FANCA	FANCC	FANCG	FANCL	FBXW7	FGF3	FGFR1	FGFR2	FGFR3	FLCN	
SNV Selected Exons							•	•	•	•	•	•	•		•						•	•	•	•	
SNV All Coding Exons				•					•		•									•				•	
CNV	•	•	•	•	•				•	•	•				•	•	•	•	•	•	•	•	•	•	•
ITD																									
Pharmacogenomic																									

	FLT3	GNAS	GNAS	GNB1	H3F3A	HRAS	IDH1	IDH2	IKZF1	JAK1	JAK2	KDR	KIT	KMT2D	KRAS	MAP2K1	MAP2K2	MAPK1	MDM2	MDM4	MEK1	MET	MILH1	MSH2	
SNV Selected Exons	•	•	•	•	•	•	•	•	•	•	•	•	•		•	•	•	•				•	•	•	•
SNV All Coding Exons															•						•		•	•	•
CNV								•				•	•		•	•	•	•	•	•	•	•	•	•	•
ITD	•																								
Pharmacogenomic																									

	MSH3	MSH6	MTOR	MYC	MYCN	MYD88	NF1	NF2	NFE2L2	NOTCH1	NPM1	NRAS	NTSC2	PALB2	PDGFRA	PIK3CA	PIK3R1	PLCG1	PMS2	POLD1	POLE	PITCH1	PTEN	PTPN11	
SNV Selected Exons			•	•		•		•		•	•	•	•	•	•	•	•	•				•	•	•	•
SNV All Coding Exons	•	•					•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
CNV	•	•		•	•		•	•	•		•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
ITD																									
Pharmacogenomic																									

	RAC1	RAF1	RBI	REL	RET	RICTOR	RUNX1	SDHB	SDHD	SETD2	SESN1	SMAD4	SMARCA4	SMARCB1	SMO	STAT3	STK11	TP53	TRAF3	TSC1	TSC2	U2AF1	VEGFA	VHL	
SNV Selected Exons	•	•			•	•		•		•					•	•					•			•	•
SNV All Coding Exons			•				•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
CNV		•	•	•			•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
ITD																									
Pharmacogenomic																									

Assay Description

Pet Name: XXXXXXXXXX

SearchLight DNA[®] detects multiple types of mutations in cancer genes:

SearchLight DNA is a Next Generation Sequencing targeted tumor-only assay that provides for the detection of single nucleotide variants (SNVs), small nucleotide insertions and deletions (indels), copy number variants (CNVs), internal tandem duplications (ITDs), and polymorphisms in tumor tissue. Genomic DNA is extracted from the patient's tumor sample. Isolated DNA is then prepared for sequencing using a custom, hybrid capture panel to enrich for target genomic regions with high actionability (Agilent). Sequencing library preparation includes shearing, purification, adaptor ligation and PCR amplification. Libraries are then clustered on a flow cell and sequenced using Illumina instruments. Sequence data are analyzed using validated bioinformatics tools (SearchLight DNA Pipeline) and canine polymorphism databases. The reference genome assembly used for alignment is CanFam 3.1. Each tumor's candidate cancer-specific mutations are queried against Vidium's proprietary knowledgebase, Vidium Insight™, which contains thousands of canine cancer biomarker associations derived from primary peer-reviewed literature to identify potential pharmacogenomic, diagnostic, prognostic, and therapeutic associations. Additionally, Vidium Insight contains human cancer biomarker associations inferred via genomic and proteomic alignments and conservation scores from the Catalogue of Somatic Mutations in Cancer (COSMIC) database. ABCB1 germline genotype is determined based on tumor-only sequencing. SNVs are reported when present at $\geq 3\%$ allele fraction. Allele fractions are dependent on tumor purity. Tumor purity is not taken into account when calculating allele fractions. Reported CNVs (gains/losses) are identified based on comparison to a copy number baseline generated from normal tissues across major breed clades and tissue types. Reported CNVs may be focal, arm-level, or chromosome-level. ITDs are reported only for KIT and FLT3 in selected exons. Pharmacogenomic polymorphisms are reported only for ABCB1 (also known as MDR1). Indeterminate results may occur due to poor sample quality or sequencing coverage, but a "qualified report" may be delivered in these below-threshold instances after a manual data review. Mean target coverage for tumor sample DNA is $\geq 200x$ (unique reads) and $\geq 89\%$ of target bases bearing $\geq 100x$ coverage.

Limitations

Samples with tumor content less than 30% may have reduced sensitivity and lead to false negative results. It is also possible that the sample contains a mutation below our established limit of detection or in a genetic region not included in our assay. Mutations present in repetitive or high GC content region or non-coding areas may not be detected. Indels larger than 40bp may not be detected. Copy number signal relative to background noise inherent in DNA from FFPE samples may affect sensitivity of reporting CNV gains/losses. The lack of a variant call does not necessarily indicate the absence of a mutation since technical limitations in some genomic regions may limit assay detection. ABCB1 germline genotype is inferred from tumor-only sequencing and it remains possible, though unlikely, that either ABCB1 loss of heterozygosity in the tumor or somatic acquisition of an ABCB1 mutation could interfere with accurate genotyping.

Disclaimers

This test was developed, and performance characteristics determined, by Vidium Animal Health. This test has not been approved by the U.S. FDA. The FDA has determined that such clearance or approval for veterinary diagnostics is not necessary. This test is used for clinical purposes for veterinary patients. It should also be noted that the data interpretations are based on our current understanding of genes and mutations and are current as of the report date. Mutations may not be listed in order of strength of evidence or appropriateness for the patient's disease. When the report does identify mutations with therapeutic implications, this does not promise or guarantee that a particular drug or treatment regimen will be effective or helpful in the treatment of disease in any patient, and the selection of any drug for patient treatment is done at the discretion of the treating veterinarian. These treatment options are based solely on published biomarker associations and do not include dosing, safety, or combinatorial guidelines. Please refer to drug labeling, published literature, and safety data for warnings, precautions, and dosing guidelines. Use caution when combining multiple drugs and be aware of potential drug interactions. Drug availability in dogs is broadly referring to their availability at a reasonable price from any of the major veterinary compounding pharmacies within the United States. Genomic mutations should be considered in the context of the patient's history, risk factors and any previous genomic testing. Variants of Uncertain Significance (VUS) may be associated with potential therapies in the future. Vidium does not update reports or send notification regarding reclassification of these mutations. Vidium Animal Health's services, including but not limited to the results contained in this report, are governed by Vidium's Terms & Conditions, which are available at vidiumah.com/terms-conditions.

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Electronically Signed By:



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