# SearchLight DNA® Clinician Report

vidium ANIMAL HEALTH

[redacted]

Phone Number: [redacted]
Account Number: [redacted]

Accession: SL23-000435
Received: 06/06/23
Reported: 06/12/23

| Pet: | Owner: | Species: | Breed: | Sex: | Age: |
|---|---|---|---|---|---|
| [redacted] | [redacted] | Canine | Golden Retriever | M/I | 8 y |

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.

## SearchLight DNA Clinician Report

Pet Name: [redacted]

## Therapeutic Biomarkers

### Treatment Options Based on Mutations

| Drug | Mutation | Available for dogs | Used in humans |
|---|---|---|---|
| **dasatinib** | PDGFRA Copy Number Gain | Yes | Yes |
| **imatinib** | PDGFRA Copy Number Gain | Yes | Yes |

### 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 , Hemangiosarcoma , Lung Carcinoma , Mammary Cancer | Yes |
| **KIT** | Copy Number Gain | Lymphoma , B-Cell Lymphoma , Hemangiosarcoma , Lung Carcinoma , Mammary Cancer , Mast Cell Tumor , Melanoma , T-Cell Lymphoma | Yes |
| **MYC** | Copy Number Gain | Lymphoma , B-Cell Lymphoma , Glioma , Head and Neck Squamous Cell Carcinoma , Hemangiosarcoma , Mammary Cancer , Melanoma , Osteosarcoma , T-Cell Lymphoma | Yes |
| **PDGFRA** | Copy Number Gain | Lymphoma , Glioma , Hemangiosarcoma , Osteosarcoma | Yes |
| **RB1** | Copy Number Loss | Histiocytic Sarcoma , Mammary Cancer , Mast Cell Tumor , Melanoma , Osteosarcoma | Yes |
| **TRAF3** | p.Val536fs | Lymphoma , B-Cell Lymphoma | Yes |

## Prognostic Biomarkers

| | | Negative Prognostic Factor in: | |
|---|---|---|---|
| Gene | Mutation | Canine cancer | Human cancer |
| **MYC** | Copy Number Gain | - | Yes |
| **PDGFRA** | Copy Number Gain | - | Yes |
| **RB1** | Copy Number Loss | - | Yes |

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: [redacted]

| Gene | ABCB1 | Mutation | No ABCB1-1Δ Mutation |
|---|---|---|---|

**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 |
|---|---|---|---|

**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 |
|---|---|---|---|

**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 |
|---|---|---|---|

**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 |
|---|---|---|---|

**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-? 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 |
|---|---|---|---|

**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 |
|---|---|---|---|

**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: [redacted]

| 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<br>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<br>Fort Collins, CO | Link |
| AAHSD005330 - F18 FDG PET/CT and Cytology for Canine Lymphoma | Colorado State University<br>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<br>Blacksburg, VA | Link |
| AAHSD005421 - Glutathione-S-Transferase Polymorphisms in Dogs | University of Wisconsin<br>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<br>Malvern, PA<br>Denver, NC | Link |
| COSU-FACC Lymphoma 1 - Drug Repurposing to Aid Treatment of Canine Lymphoma | Cornell University - College of Veterinary Medicine<br>Ithaca, NY | Link |
| COSU-FACC Lymphoma 2 - Evaluating a New Drug Combination for Dogs with Lymphoma | Cornell University - College of Veterinary Medicine<br>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<br>Malvern, PA | Link |
| OSU-CCVM Lymphoma - Canine Naive Multicentric Lymphoma Study | Oregon State University - Carlson College of Veterinary Medicine<br>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<br>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<br>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<br>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<br>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<br>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<br>Philadelphia, PA | Link |

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

## Other Clinical Trials That May Be Applicable

| | |
|---|---|
| 18 identified | See link for details |

## 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



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## 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

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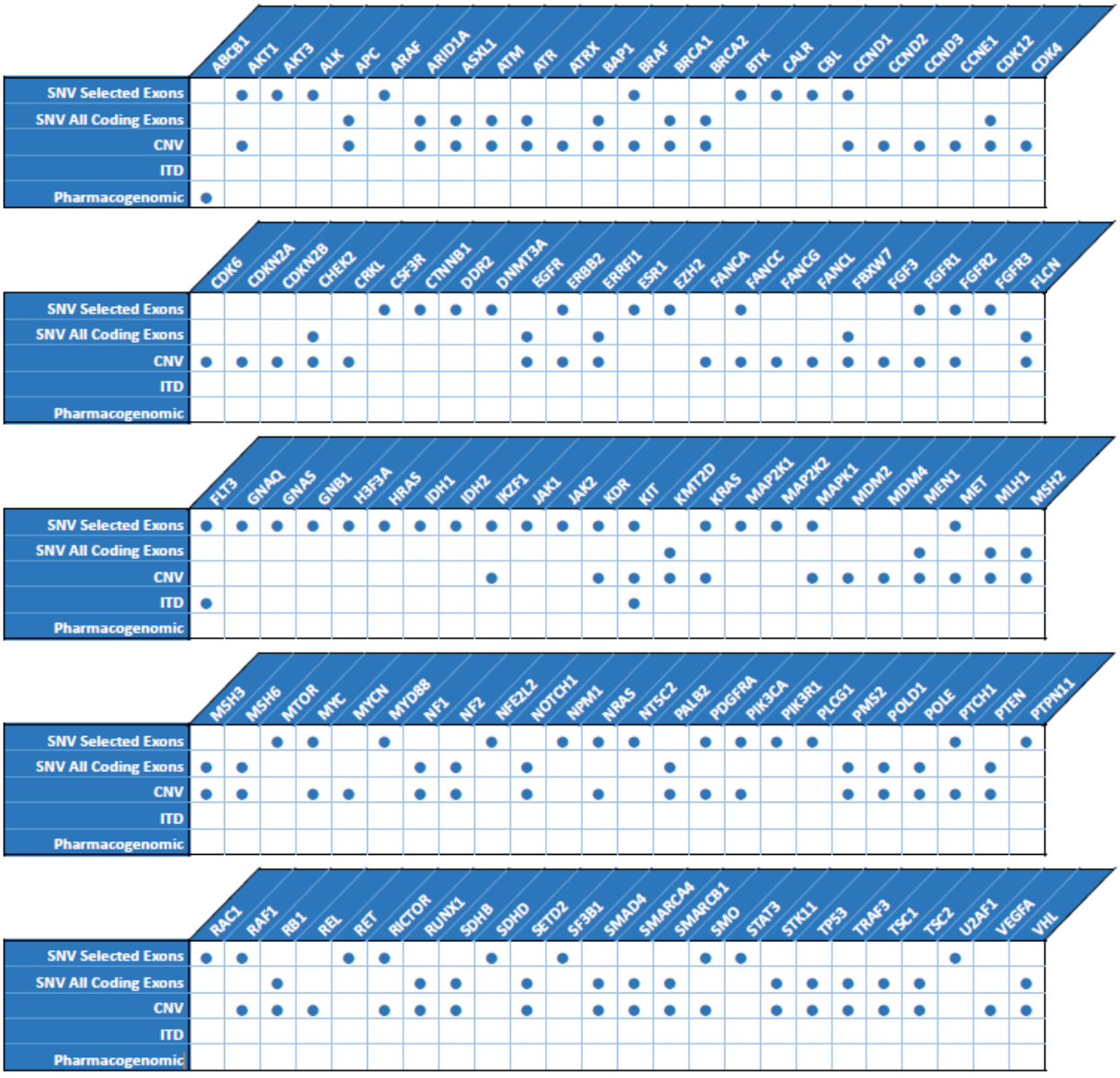
## Genes Evaluated by SearchLight DNA

Pet Name: [redacted]

**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.



## Assay Description

Pet Name: [redacted]

### 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 ≥ 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 ABCB 1 (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 ≥ 200x (unique reads) and ≥ 89% of target bases bearing ≥ 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.

### Confidential and Information Notice



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